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In this issue:

- **REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES** 1-9
- **REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES** 10-14
- **CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES** 15-16
- **CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS** 17
- **CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES** 18-23
- **PROTEIN KINASE STUDIES** 24-30

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service • National Institutes of Health • National Cancer Institute

HE 20. 3173/2:

REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES

1. GROWTH ARREST AND MORPHOLOGICAL CHANGE OF HUMAN BREAST CANCER CELLS BY DIBUTYRYL CYCLIC AMP AND L-ARGININE.

Cho-Chung YS, Clair T, Bodwin JS, Berghoffer B
Cellular Biochemistry Section, Lab. Pathophysiology, NCI, Bethesda, MD, 20205
Science; 214(4516):77-79 1981

The growth in vitro of human breast cancer cells, line MCF-7, was inhibited by a daily supplement of L-arginine (1 mg per ml). Arginine acted synergistically with dibutyryl AMP (cyclic AMP) (10(-6) M) to enhance the growth inhibitory effect: the cell replication ceased completely within 2 days after treatment. The growth arrest accompanied a change in cell morphology and was preceded by in creases in the cellular concentration of cyclic AMP, adenylate cyclase, and Type II cyclic AMP-dependent protein kinase activities as well as a decrease of estrogen binding activity. The results suggest that growth of human breast cancer cells is subject to cyclic AMP-mediated regulation and that arginine may play a specific role in this process. (Author abstract) (22 Refs)

2. TUMOR PROMOTING AGENTS.

Tsilimigras CW
Natl. Inst. for Virology, Private Bag X4, Sandringham, Johannesburg 2131, South Africa
S Afr J Sci; 77(3):100-103 1981

The effects of tumor promoting agents, particularly the phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on cells in vitro are considered. The major diterpene ester of croton oil, TPA has been shown to be the most potent promoter of multiple skin tumors in vivo. It has also been found to induce many different responses in vitro, including enhancement of transformation, stimulation of a number of specific enzymes and cyclic nucleotides, modifications in macronucleotides, modifications in macromolecular synthesis, an inhibition of terminal differentiation, changes in cellular membranes and cellular morphology, and disturbances of the transport of cations and small molecules through membranes. TPA has been reported to enhance the degree of the in vitro transformation of fibroblast cell cultures and rat embryo cells previously exposed to polycyclic aromatic hydrocarbons, UV light, or adenoviruses. TPA has also been found to enhance the expression of transformation by Rous sarcoma virus in chick embryo fibroblasts. TPA had been shown to induce ornithine decarboxylase, protease, and plasminogen activator. TPA seems to stimulate DNA and RNA synthesis. Other classes of possible tumor promoters, such as melittin, phenol, oleic acid, some non-aromatic hydrocarbons, Tween 60, saccharin, and cyclamate, are discussed. (16 Refs)

3. CAMP CONTENT IN T AND B LYMPHOCYTES IN PERIPHERAL BLOOD OF HEALTHY SUBJECTS AND PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA.

Konopka L, Karpowicz M, Brodzki M, Pawleski S
Klinika Chorob Wewnetrznych, Instytut Hematologii AM, ul. Chocimska 5, 00-791 Warsaw, Poland
Pol Arch Med Wewn; 65(4):287-293 1981

The intracellular cyclic AMP (cAMP) levels were determined in isolated peripheral T and B lymphocytes from 12 healthy blood donors and 14 patients (8 men and 6 women, 33-75 yr old) with chronic lymphocytic leukemia. The intracellular cAMP levels (in picomole/10(7) cells) were 26.70 +-4.80 in the T cells and 43.30 +-6.30 in the B cells of the donors, and significantly decreased in the leukemia patients (12.50 in the T cells and 4.50 in the B cells). After excluding the five patients in clinical Stages 0 and I, the mean cAMP levels were even lower: 10.8 in the T cells and 4.90 in the B cells. The observed decrease in the intracellular cAMP concentrations in leukemic lymphocytes is attributed to immunological and functional immaturity of the B cells. (18 Refs) (In POL)

4. ALTERATIONS TO CONTROLS OF CELLULAR DNA SYNTHESIS BY ADENOVIRUS INFECTION.

Braithwaite AW, Murray JD, Bellett AJ
Dept. Microbiology, John Curtin Sch. Medical Res., Australian Natl. Univ., Canberra, A.C.T., 2601, Australia
J Virol; 39(2):331-340 1981

Human adenovirus type 5 and temperature-sensitive mutants ts36, ts37, and ts125 induced cellular DNA synthesis in quiescent rodent cells at both permissive and nonpermissive temperatures. Cellular DNA synthesis induced by adenovirus type 5 or by serum required protein synthesis for both initiation and continuation, whereas viral DNA synthesis was not dependent upon continued protein synthesis once it was initiated. Both cellular and viral DNA replication was induced in adenovirus type 5-infected cells in the presence of dibutyryl cyclic AMP at concentrations which inhibited induction by serum, which suggested that some of the controls of DNA synthesis in serum-treated and virus-infected cells are different. After adenovirus infection of quiescent cells, there was a decrease in the number of cells with G1 DNA content and an increase in cells with G2 diploid and greater DNA contents. Thus, adenovirus type 5 induces a complete round of cellular DNA replication, but in some cells, it induces a second round without completion of a normal mitosis. These results suggest that adenovirus type 5 is able to alter cell growth cycle controls in a way which may be related to its ability to transform cells. (Author abstract) (32 Refs)

5. CHANGES IN INTERFERON AND THYMUS FACTOR LEVELS AS WELL AS IN LYMPHOCYTE POPULATIONS IN PATIENTS WITH MAMMARY CARCINOMA.

Busse E, Rose H, Helmholz M, Kaiser E, Riessbeck KH
Geschwulstklinik des Bereichs Medizin (Charite), Humboldt-Universitat, Schumannstr. 20/21, 1040 Berlin, E. Germany
Zentralbl Gynaekol; 103(12):694-702 1981

Changes in interferon and thymus factor as well as in cAMP levels and WBC populations were recorded from patients with mammary carcinoma. They were equally recordable from those in whom no clinical tumor had been detectable at the time of postsurgical examination as well as from patients with metastases distant from the primary tumor. The possibility for centralized disorder of immunological tumor cell defense in carcinoma patients is discussed. (Author abstract) (68 Refs) (In GER)

6. TWO INDEPENDENT ASPECTS OF TUMOR PROMOTION.

Berenblum I, Armuth V
Weizmann Inst. Science, Rehovot, Israel
Biochim Biophys Acta; 651(1):51-63 1981

The possibility of separate and independent mechanisms of tumor promotion is explored based on a review of evidence for sites of action within the cell. The multi-stage theory of carcinog enesis is presented. Evidence for two types of promoting action is discussed: that operating at the regulator-gene level and that operating at the level of the cell-surface membrane. Tumor promotion is generally regarded as an epigenetic phenomenon in an initiated cell which permits the phenotypic expression of an altered genotype. Since an influence on the cell's mutation rate or its repair mechanisms would need to operate close in time to initiation, these mechanisms are not widely regarded as likely. Growing evidence suggests that promotion may proceed by the derepression of a neighboring regulator gene brought about by the action of some newly synthesized tumor-specific protein(s). Cell membranes in general and cell-surface membranes in particular have an important role in regulating cellular functions, and a great variety of changes in cell-surface membranes is known to effective in this regard. Lack of cell adhesiveness and of contact inhibition in neoplastic cells have long been recognized as cell-surface phenomena. There is evidence that promoters, particularly phorbol ester promoters, can bind to cell-surface membranes and alter their properties. Induction of plasminogen activator, prostaglandins, enzymes of polyamine biosynthesis, and alterations in cyclic nucleotide levels are known cytoplasmic effects of promoter action. Implications and interrelations of these findings are discussed. (224 Refs)

7. EFFECTS OF CYCLIC-NUCLEOTIDE DERIVATIVES ON THE GROWTH OF HUMAN COLONIC CARCINOMA XENOGRAFTS AND ON CELL PRODUCTION IN THE RAT COLONIC CRYPT EPITHELIUM.

Tutton PJ, Barkla DH
Dept. Anatomy, Faculty Medicine, Monash Univ., Clayton, Victoria 3168, Australia
Br J Cancer; 44(2):182-188 1981

Previous studies have shown that various amine hormones are able to influence the growth rate of human colorectal carcinomas propagated as xenografts in immune-deprived mice, and it is now well known that the effects of many amine and other hormones are mediated by cyclic nucleotides, acting as second messengers within cells. In the present study the influence of various derivatives of cyclic AMP and cyclic guanosine monophosphate on the growth of two different lines of colorectal cancer growing in immune-deprived mice, and on the cell production rate in the colonic crypt epithelium of the rat, was assesssed. Growth of each tumor line, as well as crypt-cell production, was suppressed by treatment with N6O2' dibutyryl and N6 monobutyryl derivatives of cyclic AMP. Dibutyryl cyclic guanosine monophosphate, on the other hand, was found to promote the growth of Tumor HXK4 and to promote crypt cell production, but to have no significant effect on Tumor HXM2. (Author abstract) (35 Refs)

8. BIOLOGY OF GLIOMAS: POTENTIAL CLINICAL IMPLICATIONS OF GLIOMA CELLULAR HETEROGENEITY.

Bigner DD
Dept. Pathology, Duke Univ. Medical Center, Post Office Box 3156, Durham, NC, 27710
Neurosurgery; 9(3):320-326 1981

The morphological, cell biological, biochemical, and immunological heterogeneity of human glioma cells was explored in 11 permanent cell lines derived from anaplastic glioma, glioblastoma multiforme, gliosarcoma, and mixed anaplastic glioma. Four morphological groups were observed: fibroblastic in five cell lines, fascicular in three, epithelial in one (gliosarcoma), and glial in two. Each line, however, was sufficiently distinct morphologically to be recognized as individual. Each line was also distinct with respect to differentiation features, such as percentage of cells responding morphologically to cyclic AMP; 2'3'-cyclic nucleotide 3'-phosphohydrolase enzyme levels, glial fibrillary acidic protein expression, fibronectin levels; monoclonal antibody 81C6 binding ratios; and the expression of normal human brain-specific surface antigens. Four of the lines developed serially transplantable tumors after injection of tumor cells into athymic mice; in two others, the tumors grew initially, then regressed. It is hypothesized that the major determinant of therapeutic resistance is the genotypic and phenotypic heterogeneity of glioma cells. Evidence supporting this hypothesis is reviewed. (40 Refs)

9. DIFFERENTIAL SENSITIVITY OF NORMAL AND CHEMICALLY TRANSFORMED EPITHELIAL CELLS TO CHOLERA TOXIN.

Niles RM, Loewy B, Krah D
Dept. Biochemistry, Boston Univ. Sch. Medicine, Boston, MA, 02118
Cancer Res; 41(10):4075-4079 1981

We have been studying the regulation of growth by cyclic AMP and other factors in untransformed (K16) and chemically transformed (W8) rat liver epithelial cells. Initially, we found that 8-bromocyclic adenosine 3':5'-monophosphate was a more potent inhibitor of cell replication in K16 than in W8 cells. In addition, the phosphodiesterase inhibitor 1-methyl-3-isobutylxanthine (MIX) caused marked growth inhibition in K16 but not in W8 cells. Through the use of cholera toxin (CT) with or without MIX, we elevated intracellular cyclic AMP levels in a quantifiable fashion. With CT alone or combined with MIX, we observed a dose-dependent morphological change in W8 cells, which consisted of extensive 'process' formation. K16 morphology was not altered at any concentration of CT +- MIX tested. K16 cell growth was only marginally inhibited by CT alone, but markedly inhibited by CT plus MIX. W8 cell growth was moderately inhibited by CT alone or combined with MIX. Analysis of cyclic AMP levels revealed that, at all concentrations of CT +- MIX and at all time periods tested, W8 cells produced significantly more cyclic AMP than K16 cells. It appears that morphological changes and growth inhibition are not necessarily linked and that MIX may inhibit K16 cell replication by means other than its ability to increase intracellular cyclic AMP levels. (Author abstract) (23 Refs)

REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES

10. FURTHER EVIDENCE FOR TRANSLATIONAL REGULATION OF TYROSINE AMINOTRANSFERASE SYNTHESIS BY DIBUTYRYL CYCLIC AMP IN REUBER H35 HEPATOMA CELLS.

Snoek GT, Voorma HO, Van Wijk R
Dept. Molecular Cell Biology, Transitorium 3, Padualaan 8, 3584 CH Utrecht, The Netherlands
Biochim Biophys Acta; 655(1):107-112 1981

Cyclic AMP derivatives increase the rate of synthesis of tyrosine aminotransferase in Reuber H35 hepatoma cells. Various studies lend support to the hypothesis that cyclic AMP increases the synthesis of tyrosine aminotransferase by acting at a posttranscriptional site. The presence of a limited non-translatable pool of tyrosine aminotransferase mRNA prior to the formation of the translatable tyrosine aminotransferase mRNA implicates a possible site of action of cyclic AMP. We compared the capacity of N6,O2'-dibutyryl cyclic AMP to induce tyrosine aminotransferase synthesis when untranslatable tyrosine aminotransferase mRNA sequences are present or absent. The transition of a condition in which non-translatable tyrosine aminotransferase mRNA sequences were present to a condition in which they were absent was established by preinduction of Reuber H35 cells with dexamethasone, followed by addition of actinomycin D. In the time period thereafter, the amount of non-translatable mRNA decreased and 1.5-2 hr after addition of actinomycin D, only translatable tyrosine aminotransferase mRNA was present. It can be seen that the induction of tyrosine aminotransferase synthesis by dibutyryl cyclic AMP follows the normal decrease of tyrosine aminotransferase mRNA. We present evidence that dibutyryl cyclic AMP in Reuber H35 hepatoma cells regulates tyrosine aminotransferase synthesis at a posttranscriptional site independent of the pool of non-translatable tyrosine aminotransferase mRNA sequences, but influencing the efficiency of translation of active tyrosine aminotransferase mRNA. (Author abstract) (34 Refs)

11. INDUCTION OF COMPLEMENT RECEPTOR EXPRESSION IN CELL LINES DERIVED FROM HUMAN UNDIFFERENTIATED LYMPHOMAS. I. MODE OF ACTION OF THEOPHYLLINE AND INHIBITION BY CERTAIN PURINES.

Magrath I, Freeman C, Novikovs L
Pediatric Oncology Branch, NCI, Bethesda, MD, 20205
J Immunol; 127(3):1034-1038 1981

We have demonstrated that theophylline will induce complement-receptor expression, detected by EACM rosette formation, in three cell lines (EW36, CA46, and JD38) derived from Burkitt's lymphoma and the closely related undifferentiated lymphoma. In the present report dealing with two of these lines, we show that several methylated xanthines will induce receptor expression but that other purines or purine derivatives will not. However, purine, adenine, and adenosine inhibit the inducing effect of theophylline. Complement receptor induction is also dependent upon RNA synthesis, since actinomycin D (AD) will delay, modify, or totally inhibit receptor induction, depending upon the time of addition after theophylline and the duration of exposure. Essentially similar effects were achieved by adding AD or removing theophylline at any given time point during the first 14 hr. After 24 hr, however, whereas removal of theophylline resulted in a decline in the fraction of EACM rosette-forming cells, addition of AD did not have any effect on receptor expression, indicating that maintenance of receptors does not require continued RNA synthesis. Although purine, adenine, adenosine, and papaverine were incapable of inducing complement receptors, each was able to replace theophylline in the maintenance of receptor levels, further supporting the probability that induction and maintenance of receptors have separate mechanisms. Dibutyryl cyclic 3,5-AMP neither induced receptors, nor was it able to substitute for theophylline in the maintenance of receptors. This does not rigorously exclude the participation of cyclic nucleotides, but strengthens the likelihood that the processes of receptor induction and maintenance are not mediated by cAMP. (Author abstract) (28 Refs)

12. THE ACTIONS OF VARIOUS CYTOCHALASINS ON MOUSE ADRENAL TUMOR CELLS IN RELATION TO TROPHIC STIMULATION OF STEROIDOGENESIS.

Hall PF, Nakamura M, Mrotek JJ
Worcester Foundation for Experimental Biology, 222 Maple Ave., Shrewsbury, MA, 01545
Biochim Biophys Acta; 676(3):338-344 1981

Inhibition of the steroidogenic responses to ACTH and dibutyl cyclic AMP in Y-1 cells by four cytochalasins (E, D, B and H2B) was examined to determine whether the capacities of these agents to inhibit these responses could be related to their abilities to inhibit polymerization of actin from Y-1 cells and to their relative potencies in causing rounding of the same cells. Two steroidogenic responses were examined: production of 20alpha-dihydroprogesterone and transport of cholesterol to inner mitochondrial membrane. The relative potencies of the four cytochalasins were compared on the basis of the concentrations (ED50) required to produce half-max rate of rounding and those required to produce 50% inhibition of the steroidogenic responses to ACTH and dibutyl cyclic AMP and 50% inhibition of initial velocity of polymerization of Y-1 cell actin in 1 mM magnesium. The four cytochalasins were ranked in the same order of decreasing potency in all of these determinations (E greater than D greater than B greater than H2B). Moreover, each cytochalasin showed approx the same ED50 for all of the determinations. From these findings approx the same potency ratios were observed for the different cytochalasins in these parameters, namely E greater than D x 1.0; D greater than B x 9.3 and B greater than H2B x 1.3. These findings suggest that the cytochalasins inhibit steroidogenesis and produce rounding of cells by interfering with polymerization of actin and not as the result of effects unrelated to binding to actin. Similar results were obtained in serum-free medium. These observations lend further support to the hypothesis that the steroidogenic responses to ACTH and dibutyl cyclic AMP require microfilaments which are involved in the intracellular transport of cholesterol. (Author abstract) (28 Refs)

13. EFFECTS OF 3-ISOBUTYL-1-METHYLXANTHINE AND CYCLIC NUCLEOTIDES ON THE BIOCHEMICAL PROCESSES LINKED TO SKIN TUMOR PROMOTION BY 12-O-TETRADECANOYLPHORBOL-13-ACETATE.

Perchellet JP, Boutwell RK
McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI, 53706
Cancer Res; 41(10):3927-3935 1981

The phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX), which enhances 12-O-tetradecanoylphorbol-13-acetate (TPA)-stimulated cyclic nucleotide levels and ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) activity in mouse epidermis in vivo, was tested for its ability to modify the process of skin carcinogenesis using the initiation-promotion (two-step) model system for the production of skin tumor in mice. Topical application of 10 umol of IBMX prior to each promotion with 8.5 nmol of TPA reduced by 78% the number of papillomas per mouse. The inhibition was dose dependent. All phosphodiesterase inhibitors tested inhibited the development of skin papillomas. Topical applications of guanosine cyclic 3':5'-monophosphate and/or adenosine cyclic 3':5'-monophosphate before each treatment with TPA were also very effective in inhibiting the formation of skin papillomas. Combined treatments including IMBX, guanosine cyclic 3':5'-monophosphate and adenosine cyclic 3':5'-monophosphate reduced by 98% the incidence of skin papillomas promoted by TPA. However, IBMX treatment 24 hr after each promotion with TPA did not suppress the formation of skin papillomas. Futhermore, repeated applications of IBMX before or after initiation with 7,12-dimethylbenz[a]anthracene did not alter the development of skin tumors. These results indicate that phosphodiesterase inhibitors and cyclic nucleotides inhibit the promotion of skin papillomas. Indeed, these modifiers act on some of the biochemical events proposed to be necessary components of the carcinogenesis process. They inhibit dramatically both TPA-increased polyamine levels and TPA-stimulated RNA, protein, and DNA synthesis. Combined treatments including IBMX and cyclic nucleotides produced additive inhibitions of polyamine, RNA, protein, and DNA synthesis in relation with their greater reduction of the formation of skin papillomas. Since IBMX and cyclic AMP block the usual accumulation of putrescine and spermidine produced by TPA in mouse epidermis in vivo and inhibit the activity of ornithine decarboxylase when added in the assay mixture, these compounds may actually interact directly with the enzyme in situ as other ornithine decarboxylase inhibitors do. (Author abstract) (50 Refs)

14. EFFECTS OF 3-ISOBUTYL-1-METHYLXANTHINE AND CYCLIC NUCLEOTIDES ON 12-O-TETRADECANOYLPHORBOL-13-ACETATE-INDUCED ORNITHINE DECARBOXYLASE ACTIVITY IN MOUSE EPIDERMIS IN VIVO.

Perchellet JP, Boutwell RK
McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI, 53706
Cancer Res; 41(10):3918-3926 1981

A single topical application of 8.5 nmol of 12-O-tetradecanoylphorbol-13-acetate (TPA) to mouse skin induced rapid and transient increases in the levels of both guanosine cyclic 3':5'-monophosphate and adenosine cyclic 3':5'-monophosphate, followed by the stimulation of ornithine decarboxylase (ODC, L-ornithine carboxy-lyase, EC 4.1.1.17) activity. Peak synthesis of guanosine cyclic 3':5'-monophosphate and adenosine cyclic 3':5'-monophosphate were achieved within 10 and 60 min, respectively, while ODC activity was max stimulated between 4.5 and 6 hr. The increased levels were dose dependent and correlated with the tumor-promoting abilities of diverse compounds. However, the magnitudes of the cyclic nucleotide and ODC responses to TPA were quite different (3-to 7-and 70-fold, respectively). A single topical application of 10 umol of 3-isobutyl-1-methylxanthine (IBMX), which was able to raise the levels of cyclic nucleotides almost as much as did TPA, produced only a 9-fold increase in ODC activity. IBMX, as well as other phosphodiesterase inhibitors, enhanced the magnitude and the duration of the increases in cyclic nucleotide levels and ODC activity produced by TPA. Max stimulation of TPA-induced ODC activity was achieved when IBMX was applied within the 30-min interval preceding TPA treatment. As a result of the IBMX pretreatment, the same or higher levels of cyclic nucleotides and ODC activity could be induced with about 10x less TPA or with weak and moderate tumor promoters, as compared with the increased levels attributable to TPA alone. However, in the presence of IBMX, a discrepancy was still observed between the magnitude of the increased cyclic nucleotide response to TPA (6-to 14-fold) and the 150-fold induction of ODC activity produced by TPA. In addition, single or combined topical treatments with guanosine cyclic 3':5'-monophosphate and adenosine cyclic 3':5'-monophosphate were unable to mimic the effect of TPA on ODC activity and even depressed significantly basal and TPA-induced ODC activities in the presence of IBMX. Therefore, the findings presented in this and an accompanying paper suggest that, in mouse epidermis in vivo, if TPA-increased cyclic nucleotides participate in the molecular mechanism by which ODC activity is induced, their controlled degradation may be equally important. (Author abstract) (60 Refs)

CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES

15. ADENYLATE CYCLASE COUPLED BETA ADRENERGIC RECEPTORS OF RAUSCHER ERYTHROLEUKEMIA CELLS.

Sytkowski AJ, Kessler CJ, D'Albis JN

Div. Hematology-Oncology, Children's Hosp. Medical Center, Boston, MA

Biochem Biophys Res Commun; 101(4):1221-1227 1981

Presence of beta-adrenergic receptor-adenylate cyclase complexes in Rauscher murine erythroleukemia cells (clone R19A) was examined. Cell exposure to the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX: 3 milliM) for 15 min resulted in accumulation of 4 picomole cyclic AMP/10(6) cells. Addition of 0.1 uM (-) isoproterenol (a beta-adrenergic agonist) caused a 2.5x increase in cyclic AMP content to 10 picomole; the response was stereospecific. (-) Epinephrine at 0.1 uM increased cyclic AMP approx 2x to 7.8 picomole. Adding 0.1 uM (-) norepinephrine had no effect. Response to beta-adrenergic stimuli was dose-dependent. The activation constant for epinephrine was 8.9 x 10(-7), lower than in most systems studied. Addition of 5 x 10(-6) propranolol completely inhibited the epinephrine effect. Binding of [125I]iodohydroxybenzylpindolol (125IHYP) to the intact cells was used to measure beta-adrenergic receptor number; nonspecific binding to low affinity sites was reduced by the addition of 100 uM phentolamine, an alpha-adrenergic antagonist. Scatchard plot yielded an equilibrium dissociation constant for 125IHYP-receptor interaction of 320 picoM and a receptor density of 1,700/cell. The results document presence of a beta-adrenergic receptor-adenylate cyclase system in an erythropoietin sensitive continuous cell line. Examination of the role of beta-adrenergic stimuli in erythroid differentiation and of beta-adrenergic receptor development and interaction with adenylate cyclase are now possible. (22 Refs)

16. INTERACTION OF VASOACTIVE INTESTINAL PEPTIDE WITH A CELL LINE (HELA) DERIVED FROM HUMAN CARCINOMA OF THE CERVIX: BINDING TO SPECIFIC SITES AND STIMULATION OF ADENYLATE CYCLASE.

Prieto JC, Guerrero JM, De Miguel C, Goberna R
Dept. Biochemistry, Sch. Medicine, Sevilla, Spain
Mol Cell Biochem; 37(3):167-176 1981

The binding of vasoactive intestinal peptide (VIP) and its effect on cyclic AMP production were assessed in HeLa cells. The binding of [125I]VIP is a moderately rapid process, reversible, saturable, specific and dependent on temperature. Virtually no inactivation of the peptide is observed after 2 hr of exposure to the cells. At 15 C, the binding data obtained at steady state are compatible with the existence of two classes of binding sites: a first class with a Kd of 2.4 nM and low binding capacity (1.5 x 10(5) sites/cell) and a second class with a Kd of 100 nM and a high binding capacity (4.9 x 10(6) sites/ cell). Secretin is eight times less potent than VIP in competing with 125I VIP but glucagon, insulin and somatostatin are inactive. VIP-induced stimulation of cyclic AMP production depends on time and temperature and is potentiated by a phosphodiesterase inhibitor. A concentration of VIP as low as 10(-10) M is able to stimulate adenylate cyclase. Half-max stimulation is observed at 10(-9) M and max stimulation (4x above basal levels) at 10(-8) M VIP. Secretin is an agonist of VIP but exhibits a 1,000x lower potency with respect to adenylate cyclase activation. Glucagon, insulin and somatostatin do not show any effect. The presence of high-affinity binding sites and the high sensitivity and specificity of adenylate cyclase for VIP in HeLa cells provide a good model to study the role of this peptide on cell proliferation and differentiation. (Author abstract) (38 Refs)

CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS

17. CHARACTERIZATION OF SERUM-INDUCED ALTERATIONS IN THE CYCLIC AMP PATHWAY IN S49 LYMPHOMA CELLS.

Darfler FJ, Hughes RJ, Insel PA
Div. Pharmacology, Dept. Medicine, Univ. California, San Diego, La Jolla, CA, 92093
J Biol Chem; 256(16):8422-8428 1981

We have grown the S49 lymphoma cell line in a serum-free, chemically defined medium which contains casein, insulin, transferrin, testosterone, and linoleic acid. When grown in this medium, wild type S49 cells display a two- to three-fold increase in hormonal (beta-adrenergic, prostaglandin E1) and nonhormonal (cholera toxin) stimulation of cAMP accumulation compared to cells grown in 10% horse serum. Wild type cells grown in serum-free medium contained 30% more beta-adrenergic receptors than did serum-grown cells, although the affinity of the receptors for [3H]dihydroalprenolol was unchanged. The S49 variants, UNC and kin-, failed to show the alterations in cholera toxin- or isoproterenol-stimulated cAMP accumulation that were observed with wild-type cells. 125I-labeled cholera toxin binding studies showed no change in the number or affinity of cholera toxin receptors in wild type or kin- cells grown under the two conditions. In cell homogenates, wild type cells grown in serum-containing medium had 28-45% higher activity of cyclic nucleotide phosphodiesterase than did cells grown in serum-free medium. Growth with or without serum produced no change in the Km values (14 and 0.25 uM, respectively, for the 2 forms) of phosphodiesterase in these cells. Basal, NaF-, Gpp(NH)p-, and isoproterenol plus GTP-stimulated adenylate cyclase activities were similar in crude plasma membrane preparations from serum-free and serum-grown wild type cells. Horse serum contained a nondialyzable (greater than 8,000 daltons), nonlipid factor which depressed the isoproterenol-stimulated cAMP accumulation of wild type S49 cells and which required 3-4 hr to produce this effect. This factor app ears to act via the nucleotide-coupling unit of the adenylate cyclase system and protein kinase, and ultimately results in an increase in cyclic nucleotide phophodiesterase activity. We suggest that studies of the cAMP pathway in S49 cells should ideally be carried out under the less perturbing conditions offered by serum-free medium. (Author abstract) (35 Refs)

CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES

18. PHORBOL MYRISTATE ACETATE STIMULATION OF LYMPHOCYTE GUANYLATE CYCLASE.

Coffey RG, Hadden JW
Lab. Immunopharmacology, Memorial Sloan-Kettering Cancer Center, New York, NY, 10021
Biochem Biophys Res Commun; 101(2):584-590 1981

The effect of phorbol-12-myristate-13-acetate (PMA), a terpene diester structurally unrelated to phytohemagglutinin (PHA) or concanavalin A, on guanylate cyclase (GC) activity in human peripheral blood lymphocytes is described. Preincubation of lymphocytes with PMA stimulated both membrane and soluble forms of GC activity, measured in the presence of optimal concentrations of Ca++, Mg++, or Mn++. Values for the apparent Km (guanosine triphosphate) were reduced by PMA in the soluble fraction but not in the membranes. No significant changes in the activating effects of the cations were induced by PMA. Both membrane and soluble Mg++-dependent GC activities increased with increased PMA concentrations (0.01-10 ug/ml). Similar results were obtained with the Ca++- and Mn++-dependent enzymes. The capacity of PMA to stimulate GC activity was inhibited by the omission of Ca++, and partial (56-66%) inhibition was seen after addition of 5 milliM ethylene-bis(oxyethylene-nitrilo) tetraacetic acid. The abilities of phorbol and a series of phorbol diesters to stimulate lymphocyte GC were compared in view of the good correlation between mitogenic and tumor-promoting activities of PMA and several analogs. PMA was the most potent stimulant of GC; phorbol didecanoate, phorbol dibenzoate, and phorbol dibutyrate were of intermediate effectiveness. Phorbol diacetate caused a slight stimulation in membrane but not soluble GC, and phorbol was without effect in both enzyme preparations. (29 Refs)

19. INDUCTION OF COMPLEMENT RECEPTORS IN HUMAN CELL LINES DERIVED FROM UNDIFFERENTIATED LYMPHOMAS.

Jaffe ES, Smith SA, MaGrath IT, Freeman CB, Alabaster O, Sussman EH
Lab. Pathology, NCI, Building 10 Room 2N110, Bethesda, MD, 20205
Lab Invest; 45(3):295-301 1981

Two Epstein-Barr nuclear antigen-negative cell lines, EW36 and CA46, derived from human undifferentiated lymphomas were exposed to the methylxanthines theophylline and 3-isobutyl-1-methylxanthine. Both compounds markedly increased the proportion of cells which bears complement receptors, whereas other surface markers such as surface immunoglobulin and IgG Fc receptors were unchanged. The inducing agents hexamethylene bisacetamide and dimethylsulfoxide had a similar effect, but other compounds known to alter intracellular cyclic AMP had no effect on surface markers. The expression of complement receptors first appeared at 8 to 10 hr and was dependent upon the continued presence of theophylline in the tissue culture medium. Other effects of the methylxanthine derivatives tested included inhibition of DNA synthesis and a reduction in mean cellular vol. However, inhibition of growth per se did not result in complement receptor induction. These data suggest that the differentiation agents active in this system may be useful tools in the study of human lymphoid malignancies. (Author abstract) (22 Refs)

20. THE EFFECT OF IN VIVO DOXORUBICIN (ADRIAMYCIN) AND ACLACINOMYCIN ADMINISTRATION ON GUANYLATE CYCLASE ACTIVITY IN RAT TISSUES.

Lehotay DC, Levey BA, Rogerson BJ, Levey GS
Univ. Pittsburgh Sch. Medicine, Pittsburgh, PA, 15261
Proc Soc Exp Biol Med; 167(4):459-462 1981

The effects of doxorubicin (DOX), aclacinomycin (ACM) 4-epi-doxorubicin (E-DOX), 7-OMEN, and quelamycin (QUE) on guanylate cyclase (GC) activity in rat tissues after in vivo administration were investigated. Male Sprague-Dawley rats were injected ip with a single dose of dimethyl sulfoxide (DMSO) or one of the drugs dissolved in DMSO. With 20 mg/kg DOX, GC activity was unchanged at 4 hr, but activity in the heart was significantly depressed after 24 hr and remained so for at least 72 hr. The inhibition of GC activity in kidney, liver, as well as heart was dose dependent, in contrast to results in an in vitro study which showed significant inhibition only in homogenates from heart tissue. With ACM, GC activities remained unchanged, when compared to controls, at all times and doses tested both in heart and liver. E-DOX inhibited GC in heart, while 7-OMEN and QUE (each at 20 mg/kg) had no effect. The mechanism of the inhibition of GC by DOX and its relationship to cardiotoxicity remains unknown. (8 Refs)

21. SIGNIFICANT INCREASES OF CYCLIC AMP AND ALKALINE PHOSPHATASE IN BRONCHOALVEOLAR LAVAGE FLUIDS OF SHEEP EXPOSED TO ASBESTOS.

Lemaire I, Nadeau D, Begin R
Unite de Recherche Pulmonaire, Faculte de Medecine, Universite de Sherbrooke, Sherbrooke, Quebec J1H 5N4, Canada
Res Commun Chem Pathol Pharmacol; 33(3):567-570 1981

Exposure to asbestos for 6 mo caused a significant increase of adenosine 3',5'-cyclic monophosphate (cAMP) levels (2-fold) in bronchoalveolar lavage (BAL) fluids of sheep with no significant change in guanosine 3',5'-cyclic monophosphate (cGMP) and alkaline phosphatase (AP) activity. When exposure was prolonged for another 3 mo-period, the increase in cAMP levels persisted and was accompanied by a 4-fold stimulation of AP activity, an enzyme associated with the surfactant fraction of BAL fluids. (Auth or abstract) (10 Refs)

22. STIMULATION BY TUBULIN OF AN ADENYLATE CYCLASE FROM MURINE PLASMACYTOMA.

Simonin G, Zachowski A, Huitorel P, Pantaloni D, Paraf A
Station de Virologie ed d'Immunologie, l'Institut National Agronomique, F-78850 Thirerval-Grignon, Yvelines, France
Eur J Biochem; 118(3):515-519 1981

Different brain tubulin preparations were shown to stimulate membrane-bound adenylate cyclase from the murine plasmacytoma MOPC 173. Purified tubulin devoid of microtubule-associated proteins and of nucleoside-diphosphate kinase activity was responsible for this stimulation. Activation of the basal adenylate cyclase activity occurred in less than 2 min at 32 C and was amplified by a 4 C preincubation of tubulin with plasma membranes. Tubulin affected the Km and the V of the enzyme and was shown to be associated with the membrane during the activation phenomenon. Tubulin was more active on the basal adenylate cyclase activity than that stimulated by fluoride or guanosine 5'-[beta,gamma-imido]triphosphate. GTP has no effect on the tubulin-stimulated enzyme. (Author abstract) (44 Refs)

23. EFFECTS OF LECTINS ON CAMP PRODUCTION AND STEROIDOGENESIS IN CULTURED ADRENAL TUMOR CELLS.

Lifrak E, Chen CC, Wishnow RM
Veterans Admin. Medical Center, Long Beach, CA, 90822
Exp Cell Res; 135(1):167-177 1981

The plant lectins, concanavalin A (conA), wheat germ agglutinin (WGA), and phytohemagglutinin (PHA) stimulate steroidogenesis in cultured adrenal tumor cells. ConA max stimulated steroidogenesis at 100 ug/ml following an approx 4 hr lag phase. ConA stimulation was completely inhibited by alpha-methyl-D-mannopyranoside and the WGA effect was prevented by N-acetyl-D-glucosamine. It was also found that conA alone did not cause a measurable increase in either intra- or extracellular cyclic AMP (cAMP) production. In addition, conA when added simultaneously with ACTH doubled the intra- and extracellular cAMP production over controls treated with ACTH alone. This enhancement effect was dose dependent. When Y-1 cells were preincubated with conA and then treated with either ACTH or cholera enterotoxin (CT) there was a dose- and time-dependent inhibition of induced cAMP production. In the case of CT, the inhibitory effect occurred even with simultaneous addition of conA and CT. This effect was reversed by addition of both alpha-methyl-D-mannopyranoside and washing with Eagle's minimal essential medium (MEM) 1 hr after CT had bound to its receptor. This reversal was not apparent for the inhibitory effect of conA on ACTH-induced cAMP production which occurred after 2 h of preincubation with conA. These results demonstrate that conA, as well as the other plant lectins, interact with specific membrane receptors to reversibly stimulate steroid production as well as enhancing or inhibiting ligand-induced cAMP production in cultured adrenal tumor cells. (Author abstract) (38 Refs)

PROTEIN KINASE STUDIES

24. CYCLIC AMP-DEPENDENT PROTEIN KINASES FROM BALB 3T3 CELLS AND OTHER 3T3 DERIVED LINES.

Wehner JM, Malkinson AM, Wiser MF, Sheppard JR
Dept. Genetics and Cell Biology, Univ. Minnesota, Minneapolis, MN, 55455
J Cell Physiol; 108(2):175-184 1981

Cyclic AMP-dependent protein kinase and 3H-cyclic AMP (3H-cAMP) binding activities were compared in normal Balb 3T3 cells and SV40, chemical, and spontaneous transformants of Balb 3T3. The photoaffinity analogue of cAMP, 8-N3 cAMP, was used to measure the amounts of R-I and R-II of protein kinase subunits in sonicates obtained from cells. Analysis of protein kinase activities showed no significant differences among normal and transformed cell types. The normal 3T3 cells prepared by homogenization showed lower soluble 3H-cAMP-binding activity than the transformed strains, but higher particulate activity. Apparently the method of cell disruption affected 3H-cAMP-binding activity in 3T3 cells, but not in transformed cells. Diethylaminoethanol chromatography showed that 3T3 cells contained only R-II isozymes. The transformed cells contained both R-I and R-II isozymes. Cells from rat kidney, chicken embryo, and human and calf endothelial tissue also contained both isozymes. The 8-N3 cAMP-binding confirmed that transformed cells have much more R-I than normal 3T3 cells. Evidently the 3T3 cells were derived from embryonic precursors of adipocytes and have retained a characteristic adipocyte isozyme pattern in culture. (37 Refs)

25. PURIFIED LOW-MOLECULAR-WEIGHT PROTEIN KINASE FROM MURINE SARCOMA VIRUS PARTICLES CATALYZES TYROSINE PHOSPHORYLATION ENDOGENOUSLY BUT PHOSPHORYLATES CELLULAR PROTEINS AT SERINE.

Sen A
Dept. Cellular Biology, Scripps Clinic and Res. Foundation, La Jolla, CA, 92037
J Virol; 39(2):612-624 1981

The low-mol-wt (LMW) protein kinase associated with high-titer murine sarcoma virions has been extensively purified by ammonium sulfate fractionation, Bio-Gel P-100 gel filtration, DEAE-cellulose and carboxymethyl cellulose chromatography. The purified enzyme migrates as a 16K polypeptide in polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The enzyme catalyzes phosphotransfer with ATP as a phosphate donor to various exogenously added proteins as acceptors; it requires Mg++ and is independent of cyclic AMP. The enzyme preparation catalyzes a low level of phosphorylation in the absence of any exogenously added substrate and forms phosphotyrosine. However, in the presence of acceptor protein molecules including total soluble cytoplasmic proteins of murine sarcoma virus-transformed mouse cells, the phosphorylated end products contain predominantly phosphoserine. The virion-associated enzyme also shows a preference for phosphorylating certain polypeptides in the soluble cytoplasmic extracts of murine sarcoma virus-transformed cells. (Author abstract) (24 Refs)

26. ENDOGENOUS PROTEIN PHOSPHORYLATION IN ISOLATED PLASMA MEMBRANES OF AH-66 HEPATOMA ASCITES CELLS.

Nakajo S, Nakaya K, Nakamura Y
Lab. Biological Chemistry, Sch. Pharmaceutical Sciences, Showa Univ., Hatanodai, Shinagawa-ku, Tokyo 142, Japan
J Biochem (Tokyo); 90(1):23-31 1981

The phosphorylation of endogenous membrane proteins by an endogenous protein kinase was studied in isolated plasma membranes from AH-66 hepatoma ascites cells using [gamma-32P]ATP as a precursor. The phosphorylation occurred very rapidly in the presence of 10 mM Mg++ and reached a max level at 2 min. Ca++ strongly inhibited the phosphorylation reaction and antagonized the activation produced by Mg++. Neither cyclic AMP nor cyclic GMP had a significant effect on the phosphorylation. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography showed that only membrane proteins ranging in mol wt from 125,000 to 200,000 were heavily phosphorylated and those of less than 60,000 mol wt were not phosphorylated at all. The protein kinase activity was readily extractable from the plasma membranes with 1 mM EDTA at pH 8.5. Among exogenous substrates, the extracted protein kinase catalyzed the phosphorylation of histone, protamine and phosvitin rather than casein. When the extracted protein kinase was subjected to chromatography on DEAE-Sepharose, a single major peak of cyclic AMP-independent protein kinase was eluted at a position quite different from those of the cytosolic protein kinases. (Author abstract) (23 Refs)

27. PHOSPHORYLATION OF RIBOSOMAL PROTEINS IN HAMSTER FIBROBLASTS INFECTED WITH PSEUDORABIES VIRUS OR HERPES SIMPLEX VIRUS.

Kennedy IM, Stevely WS, Leader DP
Dept. Biochemistry, Univ. Glasgow, Glasgow G12 8QQ, Scotland
J Virol; 39(2):359-366 1981

In BHK cells infected with pseudorabies virus, there was a substantial increase in the phosphorylation of ribosomal protein S6. This increase occurred between 2 and 4 hr after infection and persisted at least until 9 hr. We estimated that in mock-infected cells S6 contained, on an av, one phosphate group per protein chain, whereas in infected cells this rose to between four and five phosphate groups per protein chain. A second ribosomal protein, either S16 or S18, was also phosphorylated after infection. No increase in cyclic AMP was found at the time of phosphorylation. We also found an increased phosphorylation of S6 in herpes simplex virus-infected BHK cells. (Author abstract) (39 Refs)

28. PROTEIN KINASES IN HELA CELLS AND IN HUMAN CERVIX CARCINOMA.

Pyerin W, Balbach N, Kubler D, Kinzel V
Institut fur Experimentelle Pathologie, Deutsches Krebsforschungszentrum, Im Neu enheimer Feld 280, D-6900 Heidelberg, W. Germany
Z Naturforsch [C]; 36(7/8):552-561 1981

Extracts of HeLa cell fractions were analyzed by DEAE- and phospho-cellulose chromatography for their range of cyclic AMP-dependent and -independent protein kinase activities phosphorylating histone and/or phosvitin; extractions were by phosphate buffered saline (soluble protein kinases) and the non-ionic detergent NP-40 (membrane-bound protein kinases). The soluble fraction contained (i) cyclic AMP-dependent histone kinase type I and cyclic AMP-dependent histone kinase type II as evidenced by their behavior on DEAE-cellulose and inhibition by the specific heat- and acid-stable protein kinase inhibitor (PKI) in a dose-related manner; both types I and II as well as their purified catalytic subunit also phosphorylated protamine and - with very low efficiency - casein but not phosvitin; (ii) a histone kinase (H), insensitive to cyclic AMP and PKI, also accepting protamine as substrate but not either casein or phosvitin; (iii) a phosvitin kinase (P), insensitive to cyclic AMP and PKI, which also phosphorylates casein but not histone or protamine. These four enzyme species were also found in NP-40 extracts of 27,000 x g residues which, however, contained further histone and phosvitin kinase activities as yet unspecified. NP-40 extracts of the microsomal fraction possessed, besides unspecified histone and phosvitin kinase activity, only the phosvitin kinase P and appeared to be devoid of histone kinases I, II, and H. The occurrence and ratios of the protein kinases classified suggest an ordered distribution over the diverse subcellular fractions of HeLa cells. The overall pattern of soluble and membrane-bound histone and phosvitin kinases in extracts of cervix carcinoma tissue, the in vivo correlate of HeLa cells, closely resembled that of similar extracts of HeLa cells. HeLa cells hence appear, despite their long in vitro history, to express protein kinase activities similar to those of their in vivo ancestors, recommending them as a subject for the study of (certain) human protein kinase systems. (Author abstract) (19 Refs)

29. PROTEIN KINASE ACTIVITY ASSOCIATED WITH EPSTEIN-BARR VIRUS-DETERMINED NUCLEAR ANTIGEN.

Kamata T, Takaki K, Hinuma Y, Watanabe Y
Dept. Molecular Biology, Tokai Univ., Sch. Medicine, Bohseidai, Isehara, Kanagawa-ken, (259-11), Japan
Virology; 113(2):512-520 1981

A cyclic AMP-independent protein kinase activity was found associated with Epstein-Barr virus-determined nuclear antigen (EBNA). The specific activity of protein kinase (casein as substrate) rose concurrently with the rise in specific activity of CF activity for EBNA during a series of chromatographic purifications on (1) Sephadex G-200, (2) DNA-cellulose, (3) DEAE-Sephadex A-50, and (4) heparin-Sepharose (or blue sepharose instead of 3 and 4). The kinase activity contained in EBNA thus obtained (about 50% pure as determined by gel electrophoresis) could be immunoprecipitated almost quantitatively with anti-EBNA-positive IgG but not with negative IgG. This kinase immunoprecipitable with antibody to EBNA was detected only in Raji cells and not in EBNA-negative lymphoblastoids, RAMOS cells, or BJAB cells. The EBNA-associated kinase in the immunoprecipitate was unable to phosphorylate exogenous casein efficiently. The analysis for in vitro phosphorylated products in the immunoprecipitate by gel electrophoresis showed that EBNA itself was phosphorylated at serine and threonine residues. (Author abstract) (24 Refs)

30. SPECTACULAR CANCER MECHANISM DOUBTED. CORNELL RETRACTS REPORTS OF KINASE CASCADE.

Stein MD
No affiliation given
Nature; 293(5828):93 1981

A Cornell University laboratory study implicated a protein kinase cascade as the mechanism by which the products of RNA tumor virus genes transform normal cells into tumor cells. Cornell and other laboratories have been unable to reproduce the results. (2 Refs)

AUTHOR INDEX WITH CITATION NUMBERS

Alabaster O 19
Armuth V 6
Balbach N 28
Barkla DH 7
Begin R 21
Bellett AJ 4
Berenblum I 6
Berghoffer B 1
Bigner DD 8
Bodwin JS 1
Boutwell RK 13
Boutwell RK 14
Braithwaite AW 4
Brodzki M 3
Busse E 5
Chen CC 23
Cho-Chung YS 1
Clair T 1
Coffey RG 18
D'Albis JN 15
Darfler FJ 17
De Miguel C 16
Freeman C 11
Freeman CB 19
Goberna R 16
Guerrero JM 16
Hadden JW 18
Hall PF 12
Helmholz M 5
Hinuma Y 29
Hughes RJ 17
Huitorel P 22
Insel PA 17
Jaffe ES 19
Kaiser E 5
Kamata T 29
Karpowicz M 3
Kennedy IM 27
Kessler CJ 15
Kinzel V 28
Konopka L 3
Krah D 9
Kubler D 28
Leader DP 27
Lehotay DC 20
Lemaire I 21
Levey BA 20
Levey GS 20
Lifrak E 23
Loewy B 9
MaGrath IT 19
Magrath I 11
Malkinson AM 24
Mrotek JJ 12
Murray JD 4
Nadeau D 21
Nakajo S 26
Nakamura M 12
Nakamura Y 26
Nakaya K 26
Niles RM 9
Novikovs L 11
Pantaloni D 22
Paraf A 22
Pawleski S 3
Perchellet JP 13
Perchellet JP 14
Prieto JC 16
Pyerin W 28
Riessbeck KH 5
Rogerson BJ 20
Rose H 5
Sen A 25
Sheppard JR 24
Simonin G 22
Smith SA 19
Snoek GT 10
Stein MD 30
Stevely WS 27
Sussman EH 19
Sytkowski AJ 15
Takaki K 29
Tsilimigras CW 2
Tutton PJ 7
Van Wijk R 10
Voorma HO 10
Watanabe Y 29
Wehner JM 24
Wiser MF 24
Wishnow RM 23
Zachowski A 22

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In this issue:

- **REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES** **1-8**
- **REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES** **9-13**
- **CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES** **14-17**
- **CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS** **18**
- **CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES** **19-20**
- **PROTEIN KINASE STUDIES** **21-27**
- **OTHER RELATED STUDIES** **28**

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REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES

1. BETA2 RECEPTOR-MEDIATED STIMULATION OF FRIEND ERYTHROLEUKEMIA CELL GROWTH BY THYROID HORMONES.

Gauwerky CE, Bersch NL, Golde DW

Div. Hematology-Oncology, Dept. Medicine, Univ. California Los Angeles Sch. Medicine, Los Angeles, CA, 90024

Exp Hematol; 9(8):821-828 1981

Thyroid hormones are known to enhance normal erythroid colony growth (CFUE) and this enhancement depends on a functional beta2-adrenergic receptor mechanism. We investigated the response of Friend cells to thyroid hormones, catecholamines, and other compounds influencing cellular cAMP activity. The thyroid hormones L-T3, L-T4, and 'reverse T3' stimulated erythroleukemia colony growth in a serum-substituted methylcellulose culture system with peak activity at 10(-7) M. Various beta-adrenergic compounds enhanced Friend leukemia colony growth; however, the alpha-adrenergic agonist phenylephrine was inactive. Dibutyl cyclic AMP and the phosphodiesterase inhibitor theophylline also enhanced Friend leukemia colony formation. Adrenergic antagonists with beta2 specificity abrogated the stimulatory effect of L-T3, L-T4, and of 'reverse T3' at equimolar concentrations. These experiments demonstrate that thyroid hormones, beta-adrenergic agonists, the phosphodiesterase inhibitor theophylline, and dbcAMP have a direct effect on the proliferation of Friend erythroleukemia cells. We conclude that thyroid hormones' action requires a functioning beta2-adrenergic receptor mechanism. Thyroid hormones directly modulate the growth of neoplastic erythroid cells in a manner consistent with their effects on normal erythropoiesis. (Author abstract) (31 Refs)

2. INHIBITION OF CELL GROWTH OF HUMAN HEPATOMA CELL LINE BEL-7402 BY DIBUTYRYL CYCLIC AMP AND AMINOPHYLLINE.

Zhang J, Liu Y

Dept. Cell Biology, Cancer Inst. Chinese Acad. Medical Sciences, Beijing, People's Republic of China

Acta Anat Sin; 12(2):187-191 1981

The effects of dibutyryl cyclic AMP and aminophylline on growth of human hepatoma (BEL-7402) cells and on their ultrastructure are described. When used alone (at 0.2 mM), both compounds inhibited cell growth; the effects increased with increasing concentrations. When used in combination, they had synergistic effects on cell growth. The inhibitory effects of the two compounds, whether used alone or in combination, were reversible. Under the combined action of the two drugs, BEL-7402 cells became elongated, with appearance of granules in the cytoplasm. Ultrastructural changes induced by these compounds included: (1) decrease in number of microvilli on cell surface; (2) increase in number of tonofibrils; and (3) loss of regularity in polyribosome distribution. These changes suggest that the inhibitory effects of dibutyryl cyclic AMP may be a result of its ability in inducing cell differentiation in BEL-7402 cells. (13 Refs) (In CHI)

3. BIOCHEMICAL SUBGROUPING OF BENIGN BREAST DISEASE TO DEFINE PREMALIGNANT POTENTIAL.

Minton JP, Abou-Issa H, Elliot JB, Foecking MK, Roseman JM, Matthews RH

Dept. Surgery, Ohio State Univ., 410 W. 10th Ave., Columbus, OH, 43210

Surgery; 90(4):652-656 1981

To determine which subgroups of benign breast disease are likely to progress to malignant degeneration, a biochemical analysis of cyclic AMP (cAMP) was run on several samples of breast tissue. cAMP is elevated in a progressive fashion from normal breast tissue to fibrosis, cystic mastitis, ductular hyperplasias, and finally to carcinoma. Correlation of cAMP levels in breast lesions with histopat hologic grading of increasing epithelial proliferation confirmed the concept that there are subgroups within those lesions that have greater malignant potential. Benign breast lesions with significantly higher cAMP levels, although still lower than those of cancer, must be considered at higher risk for eventual malignant change. (Author abstract) (19 Refs)

4. PLASMA CAMP AND CAMP-PHOSPHODIESTERASE (PDE) LEVELS IN CANCER PATIENTS BEFORE AND AFTER SURGERY.

Patel V, Banu N, Malhotra OP, Udupa KN
Surgical Res. Lab., Inst. Medical Sciences, Banaras Hindu Univ., Varanasi 221 005, India
Indian J Cancer; 18(3):181-184 1981

Plasma levels of cyclic AMP (cAMP) and cAMP-phosphodiesterase (PDE) were measured in 40 patients 1 wk before and 1 wk after surgery. Cases included ten each of liver, stomach, colon, and breast cancer. Ages ranged from 40 to 60 yr. Ten healthy individuals served as controls. Normal values were 19.614 +-0.722 picomoles (pmol) cAMP/ml plasma and 20.854 +-0.807 pmol PDE/ml plasma. All cancer patients studied showed decreased cAMP levels (11.016-14.216 pmol/ml) before surgery. PDE levels were increased (23.894-34.798 pmol/ml) in all patients before surgery. After surgery cAMP levels returned to normal in patients with breast, colon, or stomach cancer. Levels remained below normal (16.212 pmol/ml) in liver cancer patients. PDE levels decreased after surgery in all cases. Levels were normal in breast cancer patients, but remained higher than normal in the other groups. Failure of cAMP values for liver cancer patients to return to normal after surgery was probably due to the fact that liver tumors were not completely removed by surgery. Results suggest that abnormal proliferation of cancer cells may be explained by decreased cAMP and increased PDE levels. (27 Refs)

5. DISTRIBUTION OF CYCLIC 3',5'-PHOSPHODIESTERASE, MONOAMINE OXIDASE AND BETA-GLUCURONIDASE IN LIVER TUMOURS INDUCED BY TECHNICAL GRADE HEXACHLOROCYCLOHEXANE IN INBRED SWISS MICE.

Bhatt DK, Nigam SK, Lakkad BC, Aravinda Babu K, Karnik AB, Thakore KN, Kashyap SK, Chatterjee SK
Natl. Inst. Occupational Health (ICMR), Meghani Nagar, Ahmedabad 380 016, India
Indian J Exp Biol; 19(7):625-629 1981

Male inbred Swiss mice were fed 500 ppm technical grade hexachlorocyclohexane (BHC) in their diet for 8 mo after which time the distribution of cyclic 3',5'-phosphodiesterase (cPDE), monoamine oxidase (MAO), and beta-glucuronidase (bGLU) was investigated. There was no difference in mortality between the experimental and control groups. All treated animals developed hepatocellular carcinomas. The distribution of cPDE in the tumor increased, particularly in large droplet-like cytoplasmic activity. In control animals, the MAO activity in the liver was homogeneous in all areas. MAO activity in the tumor was mainly cytoplasmic and nuclei remained unstained. bGLU activity decreased considerably in tumors and non-tumor parts of treated animals compared to controls. bGLU activity was very strong in vacuoles. (24 Refs)

6. MECHANISM OF RADIOPROTECTIVE EFFECT OF BETA-MERCAPTOETHYLAMINE MEDIATED VIA THE SYSTEM OF CYCLIC NUCLEOTIDES.

Nikol'skii AV, Aripov ShM, Pushkareva NB, Romantsev EF
No affiliation given
Radiobiologiia; 21(3):363-367 1981

To evaluate the mechanism of radioprotective activity of mercaptoethylamine (MEA), (CBAxC57Bl)F1 mice received single ip injection of MEA (150 mg/kg). Within different time periods after administration, animals were sacrificed and the ratio of cyclic AMP (cAMP) and cyclic GMP (cGMP) in the liver was determined. Significant increase of cAMP:cGMP ratio and cAMP content was recorded only within 15 min after MEA administration. (21 Refs) (In RUS)

7. CHANGES OF LEUKOCYTE AND LYMPHOCYTE VALUES AND OF CYTOTOXICITY IN TUMOR-CARRYING RATS IN DEPENDENCE ON A LOCAL IRRADIATION AND INCREASE OF CAMP LEVEL, RESPECTIVELY.

Rose H, Brusse E
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Radiobiol Radiother (Berl); 22(3):331-335 1981

The effects of various therapeutic measures on aspects of immune response in tumor-bearing rats were studied. Rats of 200-250 g received 10(8) cells of Jensen sarcoma by injection into the thigh. Treatment consisted of irradiation (21 Gray, administered in 3-Gray fractions), raising of cyclic (c) (cAMP) level (by administration of antitumor substances Dopaflex (D), Amantadin (A), and measles vaccine), or a combination of the two. Radiation dose was 21 Gray, administered in 3-Gray fractions beginning 2 days after tumor cell injection. A combination of 50 mg D and 6 mg A/kg body wt was administered on the first day of treatment, with a dose of 100 mg D and 20 mg A/kg body wt on successive days. Measles vaccine (dose 10,000 TCD50/day) was administered on days 1, 3, and 5 with D and A treatment. Development of the tumor cells was accompanied by a reduction in total WBC, lymphocyte, and T-cell subpopulation counts. B-cell counts rose. The cytotoxicity of WBC against tumor cells was also reduced. These effects were dependent on tumor size. Radiation treatment was followed by a decrease in the same counts. Treatments to raise cAMP reduced the effects in animals still showing tumors, and eliminated them in animals showing no tumors after irradiation. (29 Refs) (In GER)

8. IN VITRO SCREENING OF POTENTIAL ANTI-CANCER CHEMICALS: EFFECT OF PURINE PYRIMIDINE ANALOGUES ON SEED GERMINATION.

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Toxicology; 21(3):251-260 1981

The effect of various purine and pyrimidine analogues on the germination of Cicer arietinum and Escherichia coli dihydrofolate reductase (DHFR) is reported. Initial experiments with adenine, guanine, cytosine, uracil, and thymidine (1 milliM) did not show any effect on germination at 144 hr; their analogues were quite active. Mercaptopurine stimulated germination, xanthine had no effects, and 8-azaadenine, 8-azaguanine, 2,6-diaminopurine, and 2-hydroxypurine were potent inhibitors (83-93% inhibition). 5-Aminouracil, 6-methyluracil, and methylxanthines (eg caffeine, theophylline, theobromine, and uric acid) exhibited lesser (27-44%) inhibitory effects. 5-Fluorouracil blocked germination at 144 hr to about 90%. The effect of these purine and pyrimidine analogues on RNA synthesis, judged by (2-14C)uracil incorporation, is also presented. Cyclic AMP, adenosine, indole-3-acetic acid, and folic acid partially reversed purine and pyrimidine analogue inhibition of germination. Several newly synthesized pyrimidine analogues inhibited germination and RNA synthesis. A close analogy between interference with germination and inhibition of DHFR was observed. (32 Refs)

REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES

9. RIBONUCLEOTIDE REDUCTASE IN CULTURED MOUSE LYMPHOMA CELLS. CELL CYCLE-DEPENDENT VARIATION IN THE ACTIVITY OF SUBUNIT PROTEIN M2.

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J Biol Chem; 256(18):9436-9440 1981

Ribonucleotide reductase is responsible for the production of the deoxyribonucleotides required for DNA synthesis. The enzyme is composed of two dissociable subunits, proteins M1 and M2, which are inactive alone, but are fully active when combined. From mouse S49 T lymphoma cells we have isolated and separated the two subunits and used each for determining the activity of the complementary subunit in extracts from cells of different phases in the cell cycle. Treatment of S49 cells with cAMP analogs (eg Bt2cAMP) results in the protein kinase-dependent arrest of the cells in the G1 phase of the cell cycle. Ribonucleotide reductase (holoenzyme) activity fell in S49 cells treated for more than 16 hr with Bt2cAMP but was unchanged during short term treatments. The activity of protein M2 was decreased in parallel to the overall activity of ribonucleotide reductase, while protein M1 activity changed less. Removal of Bt2cAMP after 24 hr exposure resulted in increased holoenzyme and protein M2 activities. Centrifugal elutriation of exponentially growing S49 cells separated cells into a 90% pure G1 cell population, a mixture of G1 and early S phase cells and a 95% pure S phase/G2 cell population. The specific catalytic activity of protein M1 was the same in all these fractions while that of protein M2 was decreased 60% in the G1 cell population. These results demonstrate that the ribonucleotide reduction necessary for DNA synthesis is regulated in a cell cycle-dependent fashion by the activity of the protein M2 subunit of ribonucleotide reductase. (Author abstract) (32 Refs)

10. TELEOCIDIN, A TUMOR PROMOTER, IS A POTENT PLATELET-AGGREGATING AGENT.

Kume S, Yamanaka M, Kaneko Y, Kariya T, Hashimoto Y, Tanabe A, Ohashi T, Oda T

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Biochem Biophys Res Commun; 102(2):659-666 1981

Aggregating effects of the tumor promoter teleocidin (T) were studied. Venous blood was collected from human donors who had taken no drugs for at least 1 wk. Platelets at a concentration of 300,000/ul were exposed to T at a final concentration of 10 nanog, 100 nanog, or 1 ug/ml. A weak aggregation response without release of ATP was obtained with 10 nanog T/ml. Irreversible aggregation and release of ATP occurred with 100 nanog or 1 ug T/ml. ATP release began after a lag period of approx 1 min after addition of T. Aggregation occurred without change in platelet shape. Release of [14C]-serotonin also began after a lag of 1 min and increased in a time-dependent manner. T-induced aggregation was inhibited by prostaglandin E1, dibutrylcyclic AMP, theophylline, metabolic inhibitors, or diltiazem, indicating that aggregation was dependent on platelet metabolism. Platelets from a thrombasthenic patient did not aggregate in the presence of T. Aspirin or trifluoperazine slightly reduced T-induced aggregation and almost completely eliminated ATP release. Fibrinogen was necessary for aggregation. T appeared to induce aggregation in a manner similar to ADP. (17 Refs)

11. GLUCOCORTICOID-MEDIATED INHIBITION OF ORNITHINE DECARBOXYLASE ACTIVITY IN S49 LYMPHOMA CELLS.

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Proc Natl Acad Sci USA; 78(9,part2):5669-5672 1981

Incubation of wild-type S49 lymphoma cells with glucocorticoids, such as dexamethasone and hydrocortisone, inhibits the activity of ornithine decarboxylase (L-ornithine carboxylyase, EC 4.1.1.17), the rate-limiting enzyme in the pathway of polyamine biosynthesis. The kinetics of this inhibition are more rapid than the glucocorticoid-mediated growth arrest in the G1 phase of the cell cycle or in glucocorticoid-mediated cytolysis of these cells. The inhibition of ornithine decarboxylase activity by corticosteroids is specific for steroids of the glucocorticoid class. Results obtained with variant S49 cells having lesions in the pathways of glucocorticoid or cyclic AMP action indicate that cytoplasmic glucocorticoid receptors, as well as nuclear transfer of steroid-receptor complexes, are required for the inhibition of ornithine decarboxylase activity but that this inhibition does not require hormonal activation of adenyl ate cyclase or cyclic AMP-dependent protein kinase. Because glucocorticoid-mediated inhibition of ornithine decarboxylase occurs when cellular protein synthesis has decreased less than 20%, this inhibition may represent a specific glucocorticoid-mediated deinduction of ornithine decarboxylase in S49 cells. Inhibition of ornithine decarboxylase activity may offer a useful marker for suppression of growth and cell cycle progression in these and other lymphoma cells. (Author abstract) (33 Refs)

12. INHIBITION OF IGE-MEDIATED HISTAMINE RELEASE FROM RAT BASOPHILIC LEUKEMIA CELLS AND RAT MAST CELLS BY INHIBITORS OF TRANSMETHYLATION.

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J Immunol; 127(4):1339-1344 1981

This study evaluated the effect of inhibitors of transmethylation on histamine release from rat mast cells and rat basophilic leukemia cells. IgE-mediated histamine release from rat basophilic leukemia cells (RBL-2H3 cells) was inhibited by 3-deazaadenosine (DZA) in the presence of L-homocysteine thiolactone (Hcy) or the combination of adenosine, erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), and Hcy in a dose-dependent fashion. There were no significant changes in the cellular cAMP levels by these inhibitors. Histamine release induced by anti-IgE or dextran from normal rat mast cells was also blocked by DZA plus Hcy in a dose-dependent manner. DZA at 10(-3) in the presence of 10(-4) M Hcy or the combination of 10(-3) M adenosine, 10(-4) M EHNA, and 10(-3) M Hcy inhibited lipid (perhaps phospholipid) methylation into RBL-2H3 cells without affecting choline incorporation. In the presence of 10(-3) M DZA plus 10(-4) M Hcy there was a 170-fold increase in [35S]AdoHcy with the concomitant appearance of 3-deaza-AdoHcy when the cells were incubated with [35S]methionine, thus indicating that these drugs inhibited methylation reaction(s) through the intracellular accumulation of AdoHcy and 3-deaza-AdoHcy. In contrast, histamine release from rat mast cells induced by the calcium ionophore A23187, compound 48/80, polymyxin B, or ATP was not inhibited by these compounds. These results suggest that IgE- or dextran-mediated histamine release involves methylation reaction(s), whereas the other secretagogues bypass this early step. (Author abstract) (49 Refs)

13. INSULIN SECRETION BY A TRANSPLANTABLE RAT ISLET CELL TUMOUR.

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Diabetologia; 21(3):224-229 1981

A transplantable rat islet cell tumor was studied as a possible large-scale model for insulin secretion. Stimulation and inhibition of insulin secretion by various agents were examined. Glucose (8.4-48 mM) did not stimulate secretion. Increased release of insulin was provoked by leucine (max response at 20 mM) and theophylline (max response at 5mM). Leucine and theophylline in combination produced a greater-than-additive effect. Insulin release wes also stimulated by dibutyryl cyclic AMP. Presence of glucose, pyruvate, glutamate, and fumarate in the buffer resulted in accelerated response to stimulation. Insulin release was inhibited by sodium cyanide (1 mM), antimycin A (5 uM), 2,4-dinitrophenol (0.3 mM), and the presence of lanthanum, magnesium, or cobaltous ions, adrenalin, calmodulin, and somatostatin. Insulin release by islet cell tumor was thus shown to occur in response to specific stimuli and to depend on the cellular energy system. It apparently occurs by an exocytotic process similar to that occurring in normal islet cells; the tumor may therefore be used as a model for the insulin secretory process. (39 Refs)

CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES

14. GONADOTROPIC HORMONE BINDING TO HUMAN OVARIAN TUMORS.

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Hum Pathol; 12(10):886-890 1981

A variety of normal human ovarian tissues as well as benign and malignant human ovarian neoplasms have been examined in vitro for the presence of gonadotropin binding sites and gonadotropin-stimulable cAMP production, in order to determine whether gonadotropic hormones have an effect on ovarian tumors. Binding of either FSH or hCG, or both, was demonstrated in several tumors from each of the histologic subgroups of primary ovarian tumors, including epithelial, sex cord-stromal, and germ cell types. Cyclic AMP stimulation was found in one tumor of sex cord-stromal origin, in two of epithelial origin, in two of germ cell origin, and in one tumor-like condition, pregnancy luteoma. Additional biochemical studies are needed to characterize receptor sites and to relate them to histologic features, growth behavior in vivo, and serum gonadotropin levels. (Author abstract) (15 Refs)

15. LUTROPIN RECEPTORS IN NORMAL AND TUMOUR LEYDIG CELLS.

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Biochem Soc Trans; 9(1):40-42 1981

Leydig cells from normal adult mouse and rat testes and a Leydig cell tumor can be separated into two cell populations (both containing lutropin receptors) which differ in density and steroidogenic responsiveness. The etiology of these cell types is unknown. They may reflect different phases in a cell cycle. Treatment with gonadotropin (lutropin or human choriogonadotropin) in vivo has been shown to result in the loss of lutropin receptors, a reduction in adenylate cyclase activity, and reduced stero idogenesis; treatment of rats with gonadotropin results in reduced sensitivity of Leydig cells to lutropin in vitro. Tumor cells of density 1.07 g/ml were exposed in vitro to lutropin (0.0, 10, 100, or 1,000 nanog/ml) and exhibited an initial increase in number of lutropin receptors. This reached a max in the controls after 2 hr and remained constant thereafter. All lutropin-treated cells showed decreased binding of choriogonadotropin after 1-2 hr incubation. Basal production of cyclic AMP initially reflected pretreatment with lutropin. Cyclic AMP rose sharply within 1 hr and returned to normal pretreatment levels by 4 hr. Reduction in binding of choriogonadotropin was inhibited by cycloheximide, which prevented lutropin-induced desensitization of adenylate cyclase activity. Results indicate that desensitization of Leydig cells can be induced in vitro, and loss of both lutropin receptors and adenylate cyclase activity occurs simultaneously; both processes depend on protein synthesis. (16 Refs)

16. CATECHOLAMINE-INDUCED DESENSITIZATION OF ADENYLATE CYCLASE IN RAT GLIOMA C6 CELLS. EVIDENCE FOR A SPECIFIC UNCOUPLING OF BETA-ADRENERGIC RECEPTORS FROM A FUNCTIONAL REGULATORY COMPONENT OF ADENYLATE CYCLASE.

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Building 10, Room 3-D-03, NIH, Bethesda, MD, 20205

Mol Pharmacol; 20(2):310-318 1981

C6 low passage (C6LP) cells accumulated high levels of cyclic AMP (3-4 nanomoles/mg of protein) when exposed to isoproterenol (ISO), but C6 high passage (C6HP) cells did not. Membranes of cells desensitized by prolonged exposure to ISO seemed to have lower affinity to the agonist. Dose and time dependence, effect of phosphodiesterase, and loss of beta-adrenergic receptors were examined. Adenylate cyclase activity in membranes and the relationship to NaF-stimulated activity were studied. Reaction of the different cell types to cholera toxin, activation of cyclase, and roles of NAD and phosphodiesterase inhibitor were examined. The large accumulation of cyclic AMP in C6LP cells induced by beta-agonists does not appear to mediate the subsequent desensitization process. Initial stages of catecholamine-induced desensitization in C6LP and C6HP cells seem to represent a specific uncoupling of beta-adrenergic receptor from a functional regulatory component of adenylate cyclase. (35 Refs)

17. EPINEPHRINE DESENSITIZATION OF ADENYLATE CYCLASE FROM CYC- AND S49 CULTURED LYMPHOMA CELLS.

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J Cyclic Nucleotide Res; 7(3):161-172 1981

Specific beta-adrenergic desensitization of adenylate cyclase (AC) was characterized in the AC deficient lymphoma cell line cyc- and in the wild type S49 (WT). Cells were preincubated with (-)epinephrine (EN)plus 1.0 milliM thiourea and 0.1 milliM ascorbate or with thiourea and ascorbate alone. Both cyc- and WT cells were desensitized at the same rate by 1.0 uM EN during the first 10 min after treatment. After 60 min WT cells were 75% desensitized and cyc- cells 54% desensitized. Pretreatment for 18 hr with the beta-adrenergic agonist terbutaline produced EN-stimulated desensitization of 92% in WT compared with 48% in cyc-. Beta-adrenergic receptor levels were reduced in WT from 321 to 28 femtomoles (fmol)/mg protein and in cyc- from 580 to 438 fmol/mg protein. Both WT and cyc- were resensitized by propranolol, although WT did not recover full sensitization. Half max desensitization was produced in both cell types by 0.2-0.4 uM EN, and full desensitization by 10 uM EN. Apparently both WT and cyc- undergo rapid, reversible specific beta-adrenergic desensitization of AC that is independent of the G/F proteins of the adenylate cyclase complex. A slower desensitization occurs only in WT. (27 Refs)

CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS

18. DYNAMICS OF CALMODULIN AND CYCLIC AMP PHOSPHODIESTERASE IN PLASMA MEMBRANES OF RAT LIVERS AND ASCITES HEPATOMAS.

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Biochim Biophys Acta; 677(2):174-183 1981

Plasma membranes from ascites hepatoma cells (AH-7974, AH-130) contained much smaller amounts of calmodulin (about half) and cyclic AMP phosphodiesterase (about one-third) compared to plasma membranes of rat livers. Some calmodulin molecules in liver plasma membranes were released by repeated washing. The 'washed' liver plasma membranes showed the presence of specific binding sites for externally added calmodulin molecules (bovine brain) (N = 140 pmol/mg protein Kd = 7.9 X 10(-8) M). The calmodulin content of AH-7974 plasma membranes was not reduced by repeated washing. The binding of calmodulin to the 'washed' AH-7974 plasma membranes was only of nonspecific nature with negative cooperativity. Plasma membranes (liver and AH-7974) appeared to contain both calmodulin-dependent and calmodulin-independent phosphodiesterase, but the stimulation by externally added Ca + + plus calmodulin was rather small. Externally added calmodulin-dependent phosphodiesterase (bovine brain) was bound more to 'washed' liver plasma membranes than to 'washed' AH-7974 plasma membranes. Newly bound phosphodiesterase appeared to be more sensitive to the stimulation by Ca + + plus calmodulin in 'washed' hepatoma plasma membranes than in 'washed' liver plasma membranes. Preincubation of 'washed' plasma membranes (liver and hepatoma) with calmodulin did not affect the binding of phosphodiesterase, but the sensitivity of phosphodiesterase to the stimulation by Ca + + plus calmodulin in hepatoma plasma membranes was lost. (Author abstract) (21 Refs)

CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES

19. ADENOSINE AND GUANOSINE 3',5' CYCLIC MONOPHOSPHATE PHOSPHODIESTERASE ACTIVITIES IN RAT SMALL AND LARGE BOWEL FOLLOWING SINGLE AND MULTIPLE EXPOSURES TO 1,2-DIMETHYLHYDRAZINE.

Stevens RH, Loven DP, Singh D
Dept. Radiology, Radiation Res. Lab., Univ. Iowa, Iowa City, IA. 52242
Drug Chem Toxicol; 4(2):161-172 1981

The affect of the colon carcinogen, dimethylhydrazine (DMH: 20 mg/kg, sc) on the hydrolytic activities of cyclic AMP (cAMP) and guanosine 3',5'-cyclic monophosphate (cGMP) phosphodiesterases (PDE) in the small and large intestines of adult male outbred rats was determined. Following injection of DMH, an almost immediate increase in cAMP-PDE activities in the colon tissue was observed. These elevated activites, which had increased after 1 day to greater than three times that in untreated colon, returned to normal value after approx 5 days after exposure. In contrast, the cGMP-PDE activities seemed only slightly changed by the exposure to DMH. Chronic exposures at weekly intervals to the DMH had little effect on PDE activites during the early phases of tumor activity. However, at the end of wk 20, exposed colon tissues had elevated cAMP-PDE levels similar to those in cancer cells. Approx 10-15% of DMH-treated rats developed small bowel cancer. A single exposure to DMH produced a significant increase in both the cAMP- and cGMP-PDE activities. Chronic exposure to DMH resulted in little apparent PDE changes in the small intestine until approx wk 20 at which time cAMP-PDE levels were similar to those measured in small bowel tumor cells. Ratios of cAMP and cGMP and cAMP-PDE and cGMP-PDE measured in the colon tissue following single and double DMH exposure were plotted against time. The measurements suggested that cyclic nucleotide content may be a reflection of tissue insult by an environmental toxicant. (19 Refs)

20. BIOCHEMICAL MECHANISM OF MODULATION OF SKIN CARCINOGENESIS BY RETINOIDS.

Verma AK
McArdle Lab. for Cancer Res., Univ. Wisconsin Medical Center, Madison, WI, 53706
Retinoids: Advances in Basic Research and Therapy, Proceedings of the International Dermatology Symposium held in Berlin, W. Germany, 13-15 October, 1980. Orfanos CE, Braun-Falco O, Farber EM, Grupper C, Polano MK, and Schuppli R, ed. Berlin, Springer-Verlag, 527 pp., 1981.

Investigations of mechanisms involving retinoids in the two-stage model of mouse skin carcinogenesis are reviewed. Application of retinoic acid (RA) 1 hr before application of 12-O-tetradecanoylphorbol-13-acetate (TPA) led to inhibition of induction of ornithine decarboxylase (ODC) and resultant putrescine accumulation, and inhibition of enhanced DNA synthesis at 18 hr, but not at 24 or 48 hr. RA did not inhibit S-adenosylmethionine decarboxylase induction, incorporation of [3H]leucine or [3 H]histidine into epidermal protein, induction of cyclic AMP phosphodiesterase activity, accumulation of prostaglandins E and F, or hyperplasia occurring 1 or 3 days after TPA treatment. No increase in polyamine biosynthesis was detected after topical skin treatment with 17 nanomole RA, but incorporation of [3H]thymidine increased at 42 hr. RA inhibited skin papillomas and carcinomas when administered with TPA treatments, but not when given with initiating doses of 7,12-dimethylbenz[a]anthracene (DMBA) after repeated TPA doses. RA did not promote growth of DMBA-induced tumors. Combined RA and DMBA treatment did not result in inhibition of induction of ODC or tumors. (58 Refs)

PROTEIN KINASE STUDIES

21. INCREASED PHOSPHOTYROSINE CONTENT AND INHIBITION OF PROLIFERATION IN EGF-TREATED A431 CELLS.

Gill GN, Lazar CS
Dept. Medicine, Div. Endocrinology, Univ. California San Diego, Sch. Medicine, La Jolla, CA, 92093
Nature; 293(5830):305-307 1981

The effect of epidermal growth factor (EGF) on phosphotyrosine content of exponentially growing A431 human epidermoid carcinoma cells was determined by labelling cells to equilibrium with 32P-orthophosphate, treating replicate plates 3 hr with EGF, and measuring phosphotyrosine. EGF increased phosphotyrosine content 7x from 0.02 to 0.14% of cellular phosphoamino acids, indicating that EGF activates tyrosine-specific protein kinase activity in vivo as it does in vitro and the kinase activity is an important component of the mechanism of EGF action. In contrast to Rous sarcoma virus (RSV) transformation, EGF inhibits replication of A431 cells. No growth was apparent when A431 cells were maintained with saturating concentrations of EGF over a 14-day period. Affinity of EGF receptors for EGF was inversely related to cell density. The measured equilibrium dissociation constant, Kd, for EGF binding at low and high cell densities was higher than the half-maximally effective concentration, EC50, of EGF required to inhibit cell proliferation at that cell density (7x at 500 cells/square cm and 4x at 15,000 cells/square cm). Occupancy of only a fraction of EGF receptors seems to be required for growth inhibition. Dose-response of growth inhibition was strongly affected by tissue culture substratum. (36 Refs)

22. (NA+ + K+)-STIMULATED ATPASE OF HUMAN KIDNEY, NORMAL AND ADENOCARCINOMA. PHOSPHORYLATION AND INHIBITION BY ANTITUMOR PROTEINS.

Zaheer A, Singh D, Montgomery R
Dept. Biochemistry, Univ. Iowa, Iowa City, IA, 52242
Biochem Biophys Res Commun; 102(1):86-92 1981

To evaluate the effect of antitumor proteins on metabolism of cultured tumor cells, (Na+ + K+)-stimulated ATPase isolated from normal human kidney and from renal clear cell adenocarcinoma was treated with macromomycin (MM), auromomycin (AM), largomycin (LM), and NSC 327459. MM, LM, an d NSC 327459 (all 50 ug/ml) caused 73.4-93.4% inhibition of enzyme purified from adenocarcinoma. LM and NSC 327459 produced 16.5-23.2% inhibition of the enzyme from normal kidney, but MM and AM did not affect normal kidney ATPase. AM was also ineffective against the enzyme from kidney adenocarcinoma. Cyclic AMP (5 uM) caused a 6x stimulation of the rate of phosphorylation of normal enzyme, compared with a 3x increase of phosphorylation rate for the tumor enzyme. Phosphorylation reduced the enzyme activities: the normal ATPase exhibited 28.8% of original activity and the tumor enzyme showed only 19.1%. The normal enzyme was now sensitive to MM inhibition (approx 40% at 10 ug/m). (16 Refs)

23. INDUCTION OF THE REGULATORY SUBUNIT OF TYPE I ADENOSINE CYCLIC 3':5'-MONOPHOSPHATE-DEPENDENT PROTEIN KINASE IN DIFFERENTIATED N-18 MOUSE NEUROBLASTOMA CELLS.

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Dept. Pharmacology, Harvard Medical Sch., Boston, MA, 02115
Cancer Res; 41(11,part1):4579-4587 1981

The expression of a cyclic AMP (cAMP)-binding protein, regulatory subunit of the Type I cAMP-dependent protein kinase (RI), and its functional significance in the differentiation of N-18 mouse neuroblastoma cells were examined. 8-Azidoadenosine cyclic 3':5'-[32P]monophosphate, a photoaffinity-labeling analog of cAMP, and high-resolution sodium dodecyl sulfate-polyacrylamide gel electrophoresis were used to identify and quantitate cAMP-binding proteins in cell extracts. The induction of differentiation of N-18 mouse neuroblastoma cells, initiated either by adding dibutyryl cAMP to the growth medium or by culturing cells in medium supplemented with 1% fetal calf serum, led to a three-fold increase in the amount of 8-azidoadenosine cyclic 3':5'-[32P]monophosphate incorporated into RI, when assayed in vitro. This increased incorporation was attributable to an increase in the amount of RI rather than to an increase in the affinity of RI for 8-azidoadenosine cyclic 3':5'-[32P]monophosphate. The subunit mol wt, isoelectric point, and immunoreactivity of RI were found to be identical to that of the regulatory subunit of the Type I cAMP-dependent protein kinase purified from bovine skeletal muscle. The increase in RI was not accompanied by an increase in the cAMP-dependent protein kinase activity. DEAE-cellulose column chromatography confirmed the induction of RI as a free cAMP-binding protein in the differentiated neuroblastoma cells. The possibility of a growth-dependent regulation of RI was also examined. Addition of 2% dimethyl sulfoxide to cultures of N-18 mouse neuroblastoma cells inhibited cell growth without increasing the specific activity of RI. Dimethyl sulfoxide had no effect on neurite outgrowth or acetylcholinesterase activity, two parameters characteristic of differentiated cells. The fact that the induction of RI coincided with differentiation of the neurobla stoma cells suggests that the expression of RI may be used as a biochemical index of differentiation in these cells. The presence of a free cAMP-binding protein, not associated with cAMP-dependent protein kinase in neuroblastoma cells, raises important considerations concerning the action of cAMP in the regulation of growth and differentiation. (Author abstract) (33 Refs)

24. CELL ADHESION-DEPENDENT DIFFERENCES IN ENDOGENOUS PROTEIN PHOSPHORYLATION ON THE SURFACE OF VARIOUS CELL LINES.

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Biochim Biophys Acta; 670(2):274-284 1981

Endogenous phosphorylation of intact cells was studied with four mouse, hamster and human cell lines using [gamma-32P]ATP and [gamma-32P]GTP as exogenous substrates. With all four cell lines distinct differences in the phosphoprotein patterns could be demonstrated for cells grown in suspension culture compared to cells grown in monolayers. Two major, apparently ubiquitous phosphoproteins with mol wt of 135,000 (128,000 in HeLa cells) and 105,000, representing up to 60% of total phosphorylation, were phosphorylated only in cells grown in suspension. These phosphoproteins and the kinase(s) were located on the surface of the suspension cells. Evidence showed that phosphorylation was apparently not a true endogenous reaction, that rather it occurred by cell-cell collision, showing exponentially increasing 32P incorporation with increasing cell population density. Phosphorylation of pp135 and pp105 was established with ATP as well as with GTP and was not dependent on cyclic nucleotides cyclic AMP, cyclic GMP and cyclic CMP. The substrate-attached cells of all four cell lines have protein kinases on the cell surface. The lack of pp135 and pp105 phosphorylation may be due to the fact that these phosphoproteins are not expressed at all on the surface of substrate-attached cells or that these phosphoproteins are already fully phosphorylated. (Author abstract) (32 Refs)

25. COMPARISON OF CAMP-DEPENDENT PROTEIN KINASE IN NORMAL AND ROUS SARCOMA VIRUS TRANSFORMED CHICK EMBRYO FIBROBLASTS.

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Dept. Medicine, Div. Endocrinology, Univ. California, San Diego, Sch. Medicine, La Jolla, Ca, 92093
J Cyclic Nucleotide Res; 7(3):151-159 1981

Chicken embryo fibroblasts (CEF) were transformed with Schmidt-Ruppin Subgroup D Rous sarcoma virus (RSV). The activity of cyclic AMP (cAMP) dependent protein kinase (A kinase) in transformed cells was compared with th in normal cells. Two A kinase regulatory subunits from chicken skeletal muscle, both specific for cAMP, were characterized by photoaffinity labeling. Mol wt of the units were 45,000 and 42,000 Mr, about 4-5,000 daltons smaller than corresponding mammalian subunits. A single isoenzyme corres ponding to the smaller subunit was found in equal quantities in normal and transformed CEF. Total cAMP binding and total A kinase activity were the same in normal and transformed CEF. The apparent Km for activation of A kinase by cAMP was 35 nanoM for each. Apparently RSV does not directly affect the amount or activity of cAMP-dependent A kinase. ([illegible] Refs)

26. GENETIC STRUCTURE, TRANSFORMING SEQUENCE, AND GENE PRODUCT OF AVIAN SARCOMA VIRUS UR1.

Wang L-H, Feldman R, Shibuya M, Hanafusa H, Notter MF, Balduzzi PC
Rockefeller Univ., New York, NY, 10021
J Virol; 40(1):258-267 1981

We analyzed the genetic structure and gene products of the newly isolated avian sarcoma virus UR1, which recently has been shown to be replication defective and to contain no sequences homologous to the src gene of Rous sarcoma virus. The sizes of the genomic RNAs of UR1 and its associated helper virus, UR1AV, were determined to be 29S and 35S (5.9 and 8.5 kilobases), respectively, by gel electrophoresis and sucrose gradient sedimentation. RNase T1 oligonucleotide mapping of purified viral RNAs indicated that UR1 RNA contains eight unique oligonucleotides in the middle of the genome and shares four 5'-terminal and three 3'-terminal oligonucleotides with UR1AV RNA. The unique sequences of UR1 and Fujinami sarcoma virus were found to be closely related to each other by molecular hybridization of UR1 RNA with DNA complementary to the unique sequence of Fujinami sarcoma virus RNA, but minor differences were found by oligonucleotide fingerprinting. In the regions flanking the unique sequences, UR1 and Fujinami sarcoma viral RNAs contain distinct oligonucleotides, which are shared with oligonucleotides of the respective helper viral RNAs. Cells transformed with UR1 produce a single 29S RNA species which contains a UR1 unique sequence; this species is most likely the mRNA coding for the transforming protein. In UR1-transformed cells, a phosphoprotein of 150,000 daltons (p150) was detected by immunoprecipitation with antiserum against gag proteins. p150 was associated with a protein kinase activity that was capable of phosphorylating p150 itself, Ig G of antiserum, and a soluble substrate, alpha-casein. This enzyme transferred phosphate exclusively to tyrosine residues of substrates in vitro, but p150 labeled in vivo with 32P contained both phosphoserine and phosphotyrosine. The in vitro kinase reaction was not affected by the presence of cyclic AMP or cyclic GMP and strongly preferred Mn++ over Mg++. Thus, the properties of UR1 protein are almost identical to those of Fujinami sarcoma virus protein. (Author abstract) (26 Refs)

27. CLEAVAGE OF FOUR AVIAN SARCOMA VIRUS POLYPROTEINS WITH VIRION PROTEASE P15 REMOVES GAG SEQUENCES AND YIELDS LARGE FRAGMENTS THAT FUNCTION AS TYROSINE PHOSPHOACCEPTORS IN VITRO.

Ghysdael J, Neil JC, Vogt PK
Dept. Microbiology, Univ. Southern California Sch. Medicine, [illegible] Zonal Ave., Los Angeles, CA 90033
Proc Natl Acad Sci USA; 78(9,part2):5847-5851 1981

[illegible] representing an ap- [illegible] of the defective viral genome. These polyproteins were cleaved with retrovirion protease p15. Cleavage of PRCII-p polyprotein P170, P105 of PRCII, and P140 of FSV occurred within the gag domain and generated fragments of Mr 130,000, 70,000, and 115,000, respectively, containing all of the transformation-specific sequences linked to a remnant of the original gag sequences. ESV P80 was cleaved inside the transformation-specific domain, yielding a Mr 35,000-38,000 fragment from the NH2-terminal half of the molecule consisting of the entire gag portion and some non-gag sequences and a Mr 48,000 fragment containing most of the transformation-specific sequences. The tyrosine phosphorylation sites of the polyproteins were found in every case in the transformation-specific fragments. The major serine phosphorylation site of ESV P80 was found to reside in the Mr 35,000-38,000 gag-containing fragment, probably within the transformation-specific sequences of that cleavage product. Removal of all of the gag domain of ESV P80 or most of the gag domain in PRCII-p P170, PRCII P105, and FSV P140 does not affect their ability to be phosphorylated by the polyprotein-associated tyrosine-specific protein kinase activities. This observation suggests that the gag sequences of the polyproteins of classes II (PRCII-p, PRCII, and FSV) and III (ESV) avian sarcoma viruses may not be required for this enzymatic function, which appears to be of importance in transformation. (Author abstract) (31 Refs)

OTHER RELATED STUDIES

28. IDENTIFICATION OF THE METABOLITES OF AN ANTITUMOR TRICYCLIC NUCLEOSIDE (NSC-154020).

Schweinsberg PD, Smith RG, Loo TL
(c/o Loo) Dept. Developmental Therapeutics, Univ. Texas System Cancer Center, M. D. Anderson Hosp. and Tumor Inst., Houston, TX, 77030
Biochem Pharmacol; 30(18):2521-2526 1981

The metabolism of 3-amino-1,5-dihydro-5-methyl-1-beta-D-ribofuranosyl-1, 4,5,6,8-pentaazaacenaphthylene (TCN, NSC-154020) was studied in human RBC. With use of high performance liquid chromatography, three metabolites in addition to the major metabolite. TCN 5'-monophosphate, were detected. After isolation and purification of the metabolites, their structures were elucidated by spectrometric methods and incubations with cultured cells and catabolic enzymes. All resulted from the oxidation of TCN. Two are proposed to be alpha- and beta-anomers of a D-ribofuranosyl nucleoside with a pyrimido[4,5-c]pyridazine-4-one base structure. The third metabolite is proposed to be the 5'-monophosphate of the beta-anomer. A mechanism for the oxidation of TCN is presented,

but the exact biological oxidant involved remains unclear. (Author abstract) (12 Refs)

AUTHOR INDEX WITH CITATION NUMBERS

Abou-Issa H 3
Anderer FA 24
Aravinda Babu K 5
Aripov ShM 6
Balduzzi PC 26
Banu N 4
Bersch NL 1
Bhatt DK 5
Brusse E 7
Chan T 23
Chatterjee SK 5
Chen KY 23
Chick WL 13
Clark RB 17
Cooke BA 15
Demopoulos RI 14
Dix CJ 15
Dwivedi CM 8
Elliot JB 3
Eriksson S 9
Feldman R 26
Fishman PH 16
Foecking MK 3
Friedman JA 17
Gauwerky CE 1
Ghysdael J 27
Gill GN 21
Gill GN 25
Golde DW 1
Green DA 17
Hagmann W 24
Hales CN 13
Hanafusa H 26
Hashimoto Y 10
Honeysett JM 11
Hutton JC 13
Insel PA 11
Junjappa H 8
Kammerman S 14
Kaneko Y 10
Kariya T 10
Karnik AB 5
Kashyap SK 5
Kudlow JE 25
Kume S 10
Lakkad BC 5
Lazar CS 21
Liu AY 23
Liu Y 2
Loo TL 28
Loven DP 19
Magee-Brown R 15
Malhotra OP 4
Mallorga P 16
Martin DW 9
Matthews RH 3
Minton JP 3
Montgomery R 22
Morita Y 12
Murti CR 8
Naber SP 13
Neil JC 27
Nigam SK 5
Nikol'skii AV 6
Notter MF 26
Oda T 10
Ohashi T 10
Patel V 4
Pfeifle J 24
Pushkareva NB 6
Raphael C 14
Romantsev EF 6
Rose H 7
Roseman JM 3
Ross J 14
Schweinsberg PD 28
Shibuya M 26
Singh D 19
Singh D 22
Siraganian RP 12
Smith RG 28
Sopwith AM 13
Stevens RH 19
Tallman JF 16
Tanabe A 10
Terayama H 18
Thakore KN 5
Udupa KN 4
Verma AK 20
Vogt PK 27
Wang L-H 26
Watson RK 25
Yamagami K 18
Yamanaka M 10
Zaheer A 22
Zhang J 2

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MARCH, 1982

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In this issue:

- **REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES** 1-15
- **REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES** 16-20
- **CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES** 21-30
- **CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES** 31-32
- **PROTEIN KINASE STUDIES** 33-45
- **OTHER RELATED STUDIES** 46-55

REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES

1. CYCLIC NUCLEOTIDE PHOSPHODIESTERASES IN NORMAL AND MALIGNANT HUMAN TISSUES.

Stefanovich V, Ambrus JL, Ambrus CM, Karakousis C, Takita H (c/o J. L. Ambrus), Roswell Park Memorial Inst., 666 Elm St., Buffalo, NY, 14263
J Med; 12(4):243-250 1981

Biopsy samples were obtained from tumor tissue and surrounding normal tissue from the same organs of the same patients in 25 patients. Adequate studies could be performed on all parameters in 18 tissue pairs: 12 malignant melanomas, 4

HE 20. 3173/2:

sarcomas, and 2 others. Significantly higher levels of cAMP low affinity phosphodiesterases were found in the tumors as compared to the controls. Similar differences were seen in the high affinity cAMP phosphodiesterases, but these were of borderline significance. High affinity cGMP phosphodiesterase levels did not differ significantly between normal and neoplastic tissue samples. Low affinity cGMP phosphodiesterases were not detectable in either tissues. These studies together with earlier experimental findings suggest possible auxiliary therapeutic trials with phosphodiesterase inhibitors. (Author abstract) (36 Refs)

2. PATTERNS OF CYCLIC NUCLEOTIDES AND RELATED ENZYMES IN NORMAL AND GROSS-VIRUS-TRANSFORMED RAT THYMOCYTES.

Peracchi M, Lombardi L, Bamonti-Catena F, Maiolo AT, Polli EE
Clinica Medica I, Pad. Granelli, via Francesco Sforza 35, 20122 Milan, Italy
Leuk Res; 5(4/5):413-420 1981

Cyclic nucleotide content and cyclase and phosphodiesterase activities in normal and Gross virus-transformed rat thymocytes were studied. Differences in cyclic nucleotide levels and in cyclase activities depended on how the data were expressed. Cyclic AMP (cAMP) levels were significantly lower in transformed than in normal thymocytes on the basis of protein content but no differences were apparent on the basis of cell number or DNA content. Cyclic guanosine monophosphate (cGMP) levels in transformed vs normal cells were also significantly reduced when the data were expressed per mg of protein, but significantly higher when based on cell number or DNA content. The cAMP/cGMP M ratio was 13.4 +-1.74 in transformed and 19.2 +-2.56 in normal thymocytes (p less than 0.02). Both adenylate cyclase and guanylate cyclase activities were significantly lower in transformed than in normal cells on the basis of protein content; no differences were apparent on the basis of cell number or DNA content. cAMP phosphodiesterase and cGMP phosphodiesterase activities, studied at two substrate concentrations (1 and 100 uM), were significantly higher in transformed than in normal thymocytes either per cell number, protein content, or DNA content. Normal thymocytes significantly increased cyclic nucleotide levels in response to isoproterenol, serotonin, carbamylcholine, sodium azide, and theophylline, but transformed thymocytes responded only to the latter two agents. The data indicated that cyclic nucleotide metabolism is abnormal in malignancy. (30 Refs)

3. AN INCREASE IN CALMODULIN DURING GROWTH OF NORMAL AND CANCEROUS LIVER IN VIVO.

MacManus JP, Braceland BM, Rixon RH, Whitfield JF, Morris HP
Animal/Cell Physiology Group, Natl. Res. Council Canada, Ottawa K1A OR6, Canada
FEBS Lett; 133(1):99-102 1981

Change in the calcium receptor protein, calmodulin (CaM), during growth of normal liver and a greater content of CaM in solid liver tumors is reported. Specific pathogen free liver as well as Morris hepatomas were utilized. Tissues were excised, homogenized and total, soluble, and particulate samples prepared. Radioimmunoassay for CaM was performed using 1251 labeled CaM, 2 ug anti-CaM, and standard amounts of CaM or tissue extracts. CaM content was also measured by stimulation of a cyclic-nucleotide phosphodiesterase (PDE). Results show no change in CaM content of liver after sham hepatectomy. CaM content in the soluble fraction of liver increased three fold after 68% partial hepatectomy, returning to normal after 18 hr. No change was observed in the particulate fraction of regenerating liver. Assay of CaM dependent cyclic nucleotide PDE confirmed increase of CaM during liver regeneration. The increase of CaM during liver regeneration coincides with the time when calcium is required for DNA synthesis. The relevance of the CaM rise to the calcium dependence has yet to be established. Fetal or neonatal liver had the same CaM concentration as adult liver. Various liver tumors contained more CaM than normal adult or fetal liver. The increase in CaM content in virally transformed cells in vitro is apparently a characteristic of the neoplastic state. The evidence of CaM increase during regeneration possibly indicates that CaM may be involved with the control of cell proliferation. (23 Refs)

4. LOSS OF CHROMOSOMAL HIGH MOBILITY GROUP PROTEINS HMG1 AND HMG2 WHEN MOUSE NEUROBLASTOMA AND FRIEND ERYTHROLEUKEMIA CELLS BECOME COMMITTED TO DIFFERENTIATION.

Seyedin SM, Pehrson JR, Cole RD
Dept. Biochemistry, Univ. California, Berkeley, CA, 94720
Proc Natl Acad Sci USA; 78(10):5988-5992 1981

Chromosomal high mobility group (HMG) proteins HMG1 and HMG2 from mouse neuroblastoma cells and Friend erythroleukemic cells were analyzed by acetic acid/urea/polyacrylamide gel electrophoresis. Compared to rapidly growing cells, levels of HMG1 and HMG2 were decreased in mouse neuroblastoma cells that had been induced to differentiate by serum deprivation. This comparison revealed a reciprocal relationship between these HMG proteins and H10, a histone known to be in higher concentrations in nondividing cells. When cell growth was inhibited by means of density inhibition, however, HMG1 and-2 levels were not affected in either HeLa or mouse neuroblastoma cells, even though H10 did accumulate. This observation establishes that HMG1 and-2 contents are not correlated with mitotic rate per se. Treatment of mouse neuroblastoma by sodium butyrate, which stops cell division without commitment to differentiation, had no effect on the level of HMG1 and-2. However, the level was decreased by dibutyryl cyclic AMP and dimethyl sulfoxide treatments, which, like serum deprivation, induced irreversible morphological differentiation in the neuroblastoma cells. Moreover, induction of differentiation (Hb synthesis) in Friend erythroleukemic cells by dimethyl sulfoxide showed a decrease in the contents of HMG1 and-2. These observations suggest that preferential loss of HMG1 and-2 in mouse neuroblastoma and Friend erythroleukemia cells may be related to commitment of these cells to differentiation. (Author abstract) (32 Refs)

5. HUMAN NEUROBLASTOMA, STRAIN SK-N-SH, AS A MODEL SYSTEM OF NERVE CELL DIFFERENTIATION: NEURONAL DEVELOPMENT, PLASMINOGEN ACTIVATOR RELEASE, AND UNSCHEDULED DEOXYRIBONUCLEIC ACID SYNTHESIS.

Blakely WF
University of Illinois, Urbana, IL
Diss Abstr Int [B]; 42(3):925-B 1981

In complete growth medium supplemented with 15% fetal bovine serum, SK-N-SH cells exhibited a large nucleus, with several distinct nucleoli, and neurite- and dendrite-like processes with branched end terminals. Within a few hr after subcultivation, the morphological complexity of the cells increased as evidenced by a greater number of cells with neurite processes longer than 50 um, and an increased elongation of the processes already present. The SK-N-SH cultures released progressively increasing levels of plasminogen activator activity on a per dish basis, beginning in the late-exponential phase (11 days) and extending into the post-exponential phase (23 days). To determine if DNA repair capability decreased with maturation of the cultures, unscheduled DNA synthesis (UDS) induced by UV light (UVL) was measured during the period of development of morphological complexity. With increasing morphological complexity up to day 10, the level of repair capability did not decline. The addition of 1 mM dibutyryl-adenosine 3':5'-cyclic monophosphoric acid (dibutyryl-cyclic-AMP) to the growth medium increased both the proportion of SK-N-SH cells with at least one process greater than 50 um in length (eg. approx 2-fold after 3 days of exposure) and the release of plasminogen activator activity (eg, a max of 3.8-fold on a per cell basis after 3 days of exposure). Two and a half days of treatment with 1 mM dibutyryl-cyclic-AMP did not modify the level of UVL-induced UDS in SK-N-SH cells. In addition, a preliminary study indicated that 4 1/2 days of exposure to 1 ug/ml beta-nerve growth factor (beta-NGF) resulted in an increased sprouting processes from cells, but also had no effect on repair synthesis capability. (no Refs)

6. CLONAL VARIATION IN CULTURED NEUROBLASTOMA CELLS. II. THE RELATIONSHIP OF INCREASED INTRACELLULAR CYCLIC AMP CONTENT TO INCREASED ANCHORAGE REQUIREMENT FOR GROWTH AND FLATTENED MORPHOLOGY.

Lanks KW, Lombardo JM
Dept. Pathology, State Univ. New York, Downstate Medical Center, Brooklyn, NY, 11203
J Cell Physiol; 109(1):45-51 1981

Morphology, cyclic AMP (cAMP) content, anchorage dependence, and cloning efficiency were studied in round and flattened variants of N18 murine neuroblastoma lines. Two round cell clones, N18R1 and N18R2, and two flat cell clones, N18F1 and N18F2 were derived from the parent N18 line. A flat varia nt, N18R1F1, was isolated from N18R1. Three round revertant variants, N18F1R1, N18F1R2, and N18F1R3, were isolated from N18F1. Round cells had low anchorage requirements and grew well in suspension. Flat cells had a high anchorage requirement and contained cAMP 2-5x the levels in parent round cells. Round cells treated with phosphodiesterase inhibitor at levels that did not affect the growth rate of attached cells showed a decrease in cloning efficiency to the level of attached cells. Apparently cAMP is directly related to the anchorage requirement for growth. Flat cells contained more myosin heavy chains (MyoHC) than round cells. Raising cAMP levels in round cells raised the amount of MyoHC, but not to the levels in flat cells. Apparently, although cAMP is partly responsible for MyoHC content, another process is required for full expression of increased MyoHC. Evidently cAMP is not the sole cause of flat morphology. (34 Refs)

7. CHANGES IN CYCLIC NUCLEOTIDE LEVELS DURING ERYTHROID DIFFERENTIATION IN FRIEND LEUKEMIA CELLS.

Chapman LF
Div. Biological Sciences, Univ. Missouri, Columbia, MO, 65211
Exp Cell Res; 135(2):415-418 1981

Changes in cyclic nucleotide levels were observed in Friend leukemia cells exposed to the inducers of erythroid differentiation dimethylsulfoxide (DMSO), sodium butyrate and hypoxanthine . The most extensive changes in cAMP levels occurred during the initial 24 hr growth period. In uninduced cultures, cAMP concentration increased 35-fold compared with a 7-fold increase in the presence of inducer. During the same period, cGMP levels rose about 3-fold in uninduced cells compared with a 20-fold increase in induced cells. (Author abstract) (11 Refs)

8. URINARY EXCRETION OF CYCLIC GUANOSINE 3',5'-MONOPHOSPHATE IN CHILDREN WITH MALIGNANT TUMOURS.

Puschendorf B, Grunicke H, Jentsch J, Berger H

Institut fur Medizinische Chemie und Biochemie, Universitat Innsbruck, Fritz-Pregl-Strasse 3, A-6020 Innsbruck, Austria

J Clin Chem Clin Biochem; 19(9):979-981 1981

Urinary cyclic guanosine 3',5'-monophosphate (cyclic GMP) excretion was correlated with the clinical course of malignant tumors or lymphoproliferative diseases in children 2 yr and older. Urinary cyclic GMP was measured by radioisotope dilution test with cyclic GMP binding protein; urinary levels of creatinine were determined by the picric acid method. Determinations in children with untreated malignancies showed 69/87 cyclic GMP levels greater than 1.5 micromole/g creatinine. Treated malignant tumors showed 10/51 cyclic GMP levels greater than 1.5 micromole/g creatinine, and malignant tumors in remission showed 17/48 with levels greater than 1.5 micromole/g creatinine. Follow up studies reveal GMP excretion values near normal during remission of disease; recurrences are accompanied or preceded by increased cycli c GMP excretion. Urinary cyclic GMP levels and other chemical parameters are useful in diagnosis and therapeutic response assessment in children. No correlation with size or stage of tumors is apparent. Determination of cyclic GMP excretion in adults complements other in vitro tumor tests. (12 Refs)

9. THROMBOXANES AND PROSTACYCLIN: POSITIVE AND NEGATIVE MODULATORS OF TUMOR GROWTH.

Honn KV, Meyer J

Dept. Radiation Oncology, Wayne State Univ., Detroit, MI, 48202

Biochem Biophys Res Commun; 102(4):1122-1129 1981

The effects of thromboxane A2 (TXA2), thromboxane synthetase inhibitors, and prostacyclin (PG12) on the proliferation of B16 amelanotic melanoma cells were examined. Both TXA2 and the TXA2-mimicking agent TXB2 [(15S)-hydroxy-11alpha,9alpha-(epoxymethano)prosta-5Z, 13E-dienoic acid] significantly stimulated tumor cell proliferation in a dose-dependent manner. Incubation of B16a cells with TXB2 (25 ug/ml) for 4 hr decreased the basal level of intracellular cyclic AMP and cyclic AMP released into the media without affecting cyclic GMP levels; preincubation of the cells with TXB2 (25 ug/ml) for 48 hr inhibited the increase in intracellular cyclic AMP release normally seen in response to incubation with prostaglandin E1 (PGE1: 1 ug/ml). Treatment of cultured B16a melanoma cells with either PG12 or with thromboxane synthetase inhibitors produced a significant dose-related inhibition of proliferation at noncytotoxic doses. Thromboxane synthetase inhibitors and a TXA2 receptor antagonist significantly reduced DNA synthesis by B16a cells at noncytotoxic doses. The results suggest that ther is bidirectional control of tumor cell proliferation by the balance between endogenous and/or exogenous PG12/TXA2 and that this regulation of tumor cell proliferation may center on the modulation of tumor cell cyclic AMP levels. The modulation of PG12/TXA2 ratios may be an important therapeutic locus in the control of tumor growth. (26 Refs)

10. STUDIES ON THE MECHANISM OF GANGLIOSIDE INHIBITION OF LYMPHOCYTE MITOGENESIS (MEETING ABSTRACT).

Kemp RG, Krishnaraj R
Department of Biochemistry, Chicago Medical School, North Chicago, IL, 60064
Oncodev Biol Med; AbstractsBook,1:P19 1981

This laboratory has proposed and provided evidence for the hypothesis that gangliosides shed by tumor cells inhibit normal cellular immune responses. Gangliosides, whose levels increase in the serum and malignant tissues of tumor-bearing animals and humans, bind to murine thymocytes and produce an inhibition of lectin-induced DNA synthesis. Lymphocyte cyclic AMP levels are known to be altered by various external stimuli such as mitogens, antigens and hormones; and it is generally believed that elevated intracellular cyclic AMP levels exert an overall negative influence on lymphocyte activation a nd its immune functions. To examine post-binding metabolic events in ganglioside-thymocyte interaction, we have studied the in vitro effect of tumor-associated gangliosides as well as bovine brain gangliosides on the intracellular levels of radioimmunoassayable cyclic AMP in mouse thymocytes. Commercial gangliosides (GT1, GD1a) produce a sharp increase in cyclic AMP content, an effect that is lost when the preparations are chromatographed on Florisil, a procedure known to remove ganglioside associated proteins. Such treatment does not reduce the potency of the ganglioside effect on DNA synthesis. Lesser changes in cyclic AMP content produced by Florisil purified gangliosides were observed at later time points that may coincide with the beginning of DNA synthesis. (no Refs)

11. MECHANISMS FOR INDUCTION OF DIFFERENTIATION IN HUMAN MYELOID LEUKEMIC CELL LINES (HL-60 AND U-937) (MEETING ABSTRACT).

Olsson I, Breitman TR, Keene BR
Department of Medicine, University of Lund, S-221 85 Lund, Sweden
Exp Hematol; 9(Suppl9):41 1981

In myeloid leukemia differentiation seems to be blocked at various stages of maturation. The HL-60 cell line consists predominantly of promyelocytes and is induced to differentiate to morphologically and functionally mature granulocytes by incubation with a wide variety of compounds including dimethyl sulfoxide (DMSO) and retinoic acid (RA). U-937 is a malignant monoblastic cell line. RA but not DMSO was found to induce differentiation of U-937 into monocyte like cells with the capacity to reduce nitroblue tetrazolium (NBT). In addition, mitogen stimulated mononuclear blood cells produce differentiation inducing factors (DIFs) for HL-60. Such factors are produced constitutively by some malignant T-lymphocyte lines. An activity, produced by mitogen-stimulated normal mononuclear blood cells or unstimulated malignant T-lymphocytes, cochromatographed with DIF and acts synergistically with RA to induce maturation of HL-60 and U-937. Thus the combination of 10 nM RA and DIF induced the maturation of 70-80% of HL-60 cells. The combination of 100 nM RA and DIF induced the maturation of 50% of U-937 cells. Furthermore, compounds elevating the intracellular level of cyclicAMP (cAMP) (eg dibuturyl cAMP, prostaglandins and choleratoxin) acted synergistically with RA to induce maturation of both cell lines. This maturation is, however, not dependent on the continued presence of RA as DIFs and cAMP agents are active also on cells primed for 8-16 hr with 10 nM RA. Cells primed with 10 nM RA also matured in response to exogenous ATP. There was an increase in the extent of phosphorylation of cell surface protein(s) as measured by 32P incorporation from [gamma-32P]-ATP with intact HL-60 cells previously treated for 8-16 hr with RA. (no Refs)

12. CYCLIC AMP-BINDING PROTEINS IN HUMAN MCF-7 CELLS AND BREAST CANCER (MEETING ABSTRACT).

Eppenberger U, Handloser K, Kung W, Handschin J
Department of Research and Gynecology, University Medical School, 4031 Basel, Switzerland
Oncodev Biol Med; AbstractsBook,1:54 1981

Several recent investigations have indicated that a relationship exists between intracellular levels of cyclicAMP (cAMP) and cAMP-dependent protein kinase isozymes Type I and Type II during normal and neoplastic growth. This study demonstrates a significant alteration in the cAMP-binding protein pattern (regulatory subunit R-II of Type II) of human neoplastic mammary tissue and human mammary MCF-7 cells as compared to normal and dysplastic mammary tissues. Fotoaffinity labeling of the cAMP receptors with 8-N3 [32P] cyclic AMP revealed on sodium dodecyl sulfate polyacrylamide gels 3 cAMP-binding proteins of R-II and a cAMP-binding protein apparent mol wt of 40,000, which could not be detected in normal and dysplastic mammary cells. It is suggested that the 40,000 mol wt protein results from a limited proteolysis. Only small amounts of the regulatory subunit R-I of isozyme Type I were observed in MCF-7 cells and breast cancer tissue, whereas in normal mammary tissue no R-I could be found. It is suggested that the cAMP-binding protein of 40,000 mol wt has some prognostic value with respect to endocrine therapy of breast cancer. (no Refs)

13. CAMP AND CGMP CONTENTS IN HAMSTER MELANOMAS WITH DIFFERENT GROWTH CHARACTERISTICS (MEETING ABSTRACT).

Blagoeva P, Balansky R

Institute of Oncology, Sofia, Bulgaria

Third European Workshop on Melanin Pigmentation, September 28-October 1, 1981, Prague, Czechoslovakia. Czechoslovak Medical Society 128 pp., 1981.

Recently an intensive study is being carried out on the role of cyclic nucleotides in regulation of cell proliferation and differentiation, inclusively in the case of malignant melanomas. We studied the level of cyclic nucleotides in transplanted hamster melanomas with various rates of differentiation and proliferation: a highly differentiated, pigmented and slow growing melanoma and two low differentiated (apigment one and depigmented, derived from the pigmented strain) melanomas, characterized with a more active growth rate. The pigmented strain exhibits a high tyrosinase activity, which lacks in the low differentiated melanomas. The DNA synthesis measured by the 3H-thymidine incorporation is significantly higher in apigment and depigmented tumors. The cyclic AMP (cAMP) and cyclic guanosine monophosphate levels are twice higher approx in tissues of low differentiated melanomas. Consequently to the high level of cAMP in these tumors did not correspond high rate of differentiation and low proliferation activity. (no Refs)

14. RIBONUCLEOSIDE DIPHOSPHATE REDUCTASE IN S49 MOUSE T-LYMPHOMA CELLS; CELL-CYCLE DEPENDENT VARIATION IN THE ACTIVITY OF ONE OF ITS SUBUNITS, PROTEIN M2 (MEETING ABSTRACT).

Eriksson S, Martin DW

Department of Medicine, University of California, San Francisco School of Medicine, San Francisco, CA

Thirteenth Miami Winter Symposium: Cellular Responses to Molecular Modulators, January 12-16, 1981, Miami, Florida. University of Miami 112 pp., 1981.

The cell is dependent on the activity of ribonucleotide reductase for the production of deoxyribonucleoside triphosphates necessary for DNA synthesis. This enzyme is composed of two nonidentical, loosely associated subunits--M1 and M2. We have measured the activity of the two subunits M1 and M2 separately in extracts from mouse S49 cells in different growth phases. Cells were treated with dibutyryl cyclic AMP which leads to the arrest of the cell cycle in the G1 phase. Exponentially growing cells were fractionated by centrifugal elutriation into S phase and G1 phase cells. With both methods a drastic decrease of ribonucleotide reductase activity was found in G1 cells. This effect was the consequence of decreased subunit M2 activity while subunit M1 activity was not changed during the cell cycle. (2 Refs)

15. THE GROWTH OF PRIMARY HUMAN ADRENAL CONN'S ADENOMA CELLS IS HORMONE-DEPENDENT (MEETING ABSTRACT).

Armato U, Andreis PG, Mantero F

Department of Human Anatomy, University of Padua, I-35100 Padua, Italy

In Vitro; 17(3):235 1981

Human adrenal Conn's adenomatous cells were cultured from aldosterone hypersecreting tumors according to a method used for the normal adrenal tissue. In such cultures the epithelial-like cells, exposed to Eagle's minimal essential medium added with 10% inactivated fetal bovine serum, were grouped in monolayered, homogenous colonies and were capable of spontaneous, though slow, proliferation. To identify the factors that affect the growth of these cells, 16-day-old cultures from 10 adenomata were treated for 5 days with daily doses of one of the following agents: ACTH, angiotensin II (AII), cyclic AMP, cyclic guanosine monophosphate (GMP), or with equimolar mixtures of the two hormones or cyclic nucleotides. When given singly, none of such agents could change the basal growth rate of the adenoma cells. In contrast, the mixture of cyclic AMP and GMP elicited a 3-to 4-fold increase in the fractions of cells entering S and M phases, thus doubling the number of adenoma cells within 5 days. Even the mixture of ACTH and AII promoted adenoma cell proliferation, being however only 60% as effective as the associated cyclic agents. The present results support the view that Conn's adenoma cell growth is hormonally controlled and may be mediated via both cyclic AMP and GMP; they also suggest that some other, as yet unidentified, growth factors(s), besides AII and ACTH, may be involved as well in the proliferative control of this histologically benign tumor. (1 Ref)

REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES

16. THYMIN AND CELL MEDIATED IMMUNITY.

Jin Y
Dept. Biology, Nanjing Univ., Nanjing, People's Republic of China
Shengli Kexue Jinzhan; 12(2):126-131 1981

Research on the physiological role of thymus hormones in cell-mediated immunity is reviewed. The hormones act on immature lymphocytes of the thymus and peripheral blood to transform them into the mature T-lymphocytes that take part in cell-mediated immunity of the organism. They consist of a group of polypeptides and proteins; more than 20 have been isolated and named, including thymosin, thymopoietin I, thymopoitin II, thymin, and serum thymic factor. They have been used in China as immunologic regulators combined with chemotherapy in treating immunologic deficiency syndromes, autoimmune disease, and small cell carcinoma of the lung. Results so far have been very promising. The analysis of thymus hormones, the roles of cyclic nucleotides (cAMP and cGMP), and the role of aging in relation to the immune system are described. (48 Refs) (In CHI)

17. PLATELETS, PROSTAGLANDINS AND THROMBOTIC DISORDERS.

de Gaetano G
Lab. Cardiovascular Clinical Pharmacology, Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea, Milano, Italy
Clin Haematol; 10(2):297-326 1981

Platelet function is modulated by factors including Ca^{++} availability, cyclic AMP, dietary and plasma factors, chemicals, and drugs. Altered platelets and vascular prostaglandins may contribute to thrombosis in disorders such as diabetes mellitus, ischemic heart disease, nephrotic syndrome, thrombotic microangiopathy, toxemia of pregnancy, and myeloproliferative disorders (including chronic myeloid leukemia), as well as during contraceptive treatment with steroid drugs. (132 Refs)

18. IMMUNOCYTOCHEMICAL STUDIES OF MEMBRANE PROTEINS IN LYMPHOID CELLS.

Butman BT
Wayne State University, Detroit, MI, 48202
Diss Abstr Int [B]; 42(4):1271-B 1981

Polycationized ferritin (PCF), a non-conventional ligand was tested for its ability to induce the patching and capping of anionic molecules on the surface of murine T-lymphoma cells. Binding of PCF at 0 degree for 1 hr induces the appearance of patches whereas subsequent incubation at 37 degrees to 60 min causes the formation of a cap structure, a process which has been found to be temperature sensitive and to require metabolic energy and an intact cytoskeletal system. Using a double immunofluorescence technique, it has been observed that the PCF-induced cap coincides with an accumulation of intracellular actin directly beneath the cap structure. Therefore PCF, behaves very much like certain conventional multivalent ligands with respect to its ability to induce receptor redistributions on the surface of lymphoid cells. Cyclic nucleotides were tested for their effect on capping induced by PCF, Concanavalin A, and monoclonal antibodies against the lymphocyte membrane glycoproteins T-200, Thy-1, and gp 69/71. It was found that agents which are known to elevate intracellular cyclic

AMP (cAMP) levels cause a remarkable stimulatory effect on receptor capping induced by all ligands tested. (no Refs)

19. DIFFERENTIATION OF SECRETORY RESPONSES IN PANCREATIC ACINAR CARCINOMA (MEETING ABSTRACT).

Warren JR
Department of Pathology, Northwestern University Medical School, Chicago, IL, 60611
Newsletter Natl Pancreas Cancer Project; VI(2):64 1981

Functional differentiation of pancreatic acinar carcinoma of rat has been asessed by comparing responsiveness of the acinar carcinoma and normal rat pancreas to four different secretagogues. Protein secretion was measured as the energy-dependent release by acinar carcinoma fragments or normal pancreas lobules of 3H-leucine labeled protein and amylase. The following observations were made: (1) the maximal rates of carbamylcholine- and cholecystokinin octapeptide-induced protein secretion by carcinoma fragments were one-third to one-half the maximal rates determined for normal pancreas lobules; (2) the Ca++ ionophore A23187 stimulated protein secretion by the acinar carcinoma to maximal rates 2-3 fold greater than those obtained with the specific cholinergic and peptide secretagogues, and comparable to ionophore-induced rates observed with normal pancreas; (3) unlike normal pancreas lobules, acinar carcinoma fragments were refractory to stimulation with adenosine 3',5'-cyclic monophosphate. Adult levels of A23187-induced protein secretion coupled with relatively low responses to cholinergic and peptide secretagogues are reminiscent of near-term embryonic rat pancreas. However, unlike the acinar carcinoma, embryonic pancreas is also highly responsive to adenosine 3',5'-cyclic monophosphate. Thus, functional differentiation in the acinar carcinoma appears not to precisely mimick functional differentiation during embryogenesis of normal rat pancreas. Additional work is required to define specific mechanisms (transmembrane Ca++ fluxes, cyclic nucleotide levels) for the aberrant differentiation apparent in the observed secretory properties of acinar carcinoma. (1 Ref)

20. DESENSITIZATION TO GONADOTROPINS IN CULTURED LEYDIG TUMOR CELLS INVOLVES LOSS OF GONADOTROPIN RECEPTORS AND DECREASED CAPACITY FOR STEROIDOGENESIS.

Freeman DA, Ascoli M
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Proc Natl Acad Sci USA; 78(10):6309-6313 1981

The ability of human choriogonadotropin (hCG) to regulate its receptors and target cell responses has been studied in a clonal strain of cultured Leydig tumor cells (MA-10). Exposure of t he MA-10 cells to hCG results in decrease in hCG binding activity which is dependent on time and the concentration of hCG. This decrease is due to a change in the number of receptors rather than in the affinity of the receptors, and it is accompanied by a corresponding reduction in the ability of hCG to stimulate steroidogenesis. Exposure of the MA-10 cells to hCG also resulted in a reduction of the steroidogenic responses to cholera toxin and 8-Br-cyclic-AMP. The hCG-induced loss of steroidogenic responses to these stimuli seems to be due to the stimulation of steroidogenesis rather than to the decrease in hCG receptors because it also can be induced when steroidogenesis is stimulated with cholera toxin or 8-Br-AMP under conditions such that the number of hCG receptors is not reduced. (Author abstract) (21 Refs)

CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES

21. ASSOCIATION OF A 68,000-DALTON PROTEIN WITH ADRENOCORTICOTROPIN-SENSITIVE ADENYLATE CYCLASE ACTIVITY IN Y1 ADRENOCORTICAL TUMOR CELLS.

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J Biol Chem; 256(21):11365-11371 1981

This report explores the biochemical basis for clonal variation in adrenocorticotropin (ACTH)-sensitive adenylate cyclase activity in the Y1 mouse adrenocortical tumor cell line. We demonstrate that the level of a specific protein, designated p68, is significantly correlated with the ability of adrenocorticotropin to stimulate adenylate cyclase activity among Y1 subclones ($p = 0.004$; $r = 0.65$). p68 was characterized by its mol wt in sodium dodecyl sulfate polyacrylamide gels ($M_r = 68{,}000$) and by its isoelectric point as determined by two-dimensional gel electrophoresis ($pI = 7.2$). On two-dimensional gels, the protein migrated as a major spot with satellite spots 0.1 pH unit on either side. Homogenates and plasma membrane fractions from clones highly responsive to ACTH had large amounts of p68. In homogenates from highly responsive clones, p68 represented 10 to 12% of the total protein. Homogenates and plasma membrane fractions from clones insensitive to ACTH were deficient in p68. In homogenates from the insensitive clones Y6 and OS3, p68 represented less than or equal to 0.8% of the total protein. A somatic cell hybrid, formed by fusion of these two ACTH-insensitive clones recovered ACTH-sensitive adenylate cyclase activity and concomitantly expressed appreciable levels of p68. It is suggested that p68 may regulate the transfer of information from the occupied ACTH receptor to the catalytic subunit of adenylate cyclase. (Author abstract) (28 Refs)

22. ADENOCARCINOMA OF THE HUMAN EXOCRINE PANCREAS: PRESENCE OF SECRETIN AND CAERULEIN RECEPTORS.

Estival A, Clemente F, Ribet A

INSERM U 151 - CHU Rangueil Bat L3, 31054 Toulouse Cedex, France

Biochem Biophys Res Commun; 102(4):1336-1341 1981

The nucleotide response of a human pancreatic adenocarcinoma transplanted in athymic nude mice was examined after stimulation with cerulein (CR) and secretin. Secretin (10(-6) M) increased the cyclic AMP level in the cancer cells, with a half-maximal response obtained at a concentration of 3 x 10(-7) M. CR increased the cyclic GMP level without affecting the cyclic AMP level. The CR concentration necessary to obtain a half-maximal response was approx 5 x 10(-11) M. The kinetics of the cyclic AMP and cyclic GMP responses were similar. The maximal nucleotide concentration was found at approx 3 min and never increased to greater than 2x the basal value, in spite of the presence of phosphodiesterase inhibitors. These results demonstrate the existence of secretin and CR receptors in adenocarcinoma of the human exocrine pancreas. (15 Refs)

23. DIRECT BINDING STUDIES OF ADENOSINE RECEPTORS.

Schwabe U
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Trends Pharmacol Sci; 2(11):299-303 1981

Classification of adenosine receptor subtypes is discussed and development of radioligands for adenosine receptors is summarized. Stimulation and inhibition of adenylate cyclase by various tissues is presented in tabular form. Present data show that efforts to identify and characterize adenosine receptors by direct ligand binding are just beginning but such studies have provided a new approach to their study. (20 Refs)

24. COMPARISON OF HORMONE RESPONSES BETWEEN CULTURED CELLS AND XENOGRAFTS FROM THE SAME HUMAN PANCREATIC CANCER (MEETING ABSTRACT).

Clemente F, Estival A, Trocheris V, Hollande E, Ribet A
INSERM U 151, CHU Rangueil, 31400 Toulouse, France
Newsletter Natl Pancreas Cancer Project; VI(2):16 1981

The purpose of the present study was to compare the hormone-evoked response of tumor cells heterotransplanted into athymic mice to that of the same cells established in continuous cell culture. The cultured cells were dissociated by treatment with a soln of trypsin (0.1%). The tryptic activity was then inhibited by serum trypsin inhibitor. All the experiments were performed incubating cells in medium 199 (Hank's soln) containing 15 mM Hepes, 1% albumin and 5 mM theophyllin. The tumor cells maintained 'in vivo', were isolated from xenografts and incubated in the same conditions. Caerulein stimulation evokes a comparable cyclic guanosine monophosphate rise in 'in vivo' and in cultured cells. The cAMP increase produced by the vasoactive intestinal peptide (VIP) stimulation of the cultured cancer cells is 10-15 x higher than that of 'in vivo' cells, whereas the secretin-and the dopamine-evoked responses of cultured cells are only three x higher. The dose-response curves of VIP and secretin are unmodified, the half-maximal stimul ation of 'in vivo' and in culture' cells is elicited by 2.10(-9) M VIP and 6.10(-7)7 M secretin. (no Refs)

25. PROSTAGLANDIN E2 (PGE2) IN TUMORS AND ANTAGONISTS OF ITS EFFECTS (MEETING ABSTRACT).

Ambrus JL, Ambrus CM, Attallah A, Lee JB, Stefanovitch V, Karakousis C
Roswell Park Memorial Institute and the State University of New, York at Buffalo Buffalo, NY
International Conference on Prostaglandins and Cancer, August 30-September 2, 1981, Washington, DC. Lombardi Cancer Center 75 pp., 1981.

Biopsies were obtained from cancer patients from the tumors and surrounding normal tissue. Tumor tissue contained higher levels of PgE2 and higher levels of cyclicAMP (cAMP) phosphodiesterases, particularly of the low affinity types. PgE2 may represent a 'tumor against host defense', since it interferes with cytotoxicity. PgE2 also induces platelet aggregation and increases rigidity of RBC. It thus may contribute to the impairment of the abnormal microcirculation of solid tumors. We have found in the same patients decreased RBC deformability using the method of Reid et al. PgE2 also produces osteoporosis and hypercalcemia. Drugs which inhibit phosphodiesterases and increase prostacyclin (Pgi2) synthesis were found to inhibit these effects. Pentoxiffline (Px), a methylxanthine derivative and RA 233, a pyrimido-pyrimidine derivative increased Pgi2 levels, inhibited cAMP phosphodiesterases, deformability and inhibited experimental osteoporosis in heparin treated mice. These agents were shown to inhibit spontaneous metastasis in transplanted C1300 neuroblastoma in A/J mice and Furth-Columbia Wilms' Tumor in Furth-Wistar rats. (2 Refs)

26. THROMBOXANES AND PROSTACYCLIN: POSITIVE AND NEGATIVE MODULATORS OF TUMOR CELL PROLIFERATION (MEETING ABSTRACT).

Honn KV, Meyer J, Dunn JR
Wayne State University, Detroit, MI, 48202
International Conference on Prostaglandins and Cancer, August 30-September 2, 1981, Washington, DC. Lombardi Cancer Center 75 pp., 1981.

Prostacyclin (PGI2) and thromboxane A2 (TxA2) are potent short lived bioregulators which reciprocally regulate cyclic AMP (cAmp) levels in platelets. Their role in regulation of tumor cell proliferation was investigated in elutriated, solid tumor derived and cultured Lewis lung carcinoma (3LL) and B16 amelanotic melanoma (B16a) cells. Two thromboxane synthetase inhibitors (U51605, U54701) and a TxA2 receptor antagonist (U54874) inhibit DNA synthesis and in vitro cell proliferation in 3LL and B16a cells at non-cytotoxic doses. Exogenous TxB2 stimulates B16a cell proliferation 300% over 7 days at 25 ug/ml. The TxA2 mimicking agent (U46619) stimulates B16a cell proliferation 54% at 1 ug/ml x 7 dys. PGE1 treated B16a cells produce a rapid (15 min) release of cAMP into the media. TxB2 pre-treated cells challenged with PGE1 have a decreased response. Prostacyclin produces a dose dependent increase in cAMP levels in B16a and inhibits their proliferation in vitro. Collectively these results suggest a role for intracellular and extracellular TxA2 and PGI2 in the regulation of tumor cell proliferation and indicate a possible locus for therapeutic intervention. (no Refs)

27. DISTRIBUTION OF PROSTAGLANDINS, B, E AND F SERIES IN PLASMA OF CANCER PATIENTS (MEETING ABSTRACT).

Hokama Y, Harvey PC, Cripps C, Kimura LH
Cancer Center, University of Hawaii, Honolulu, HI
International Conference on Prostaglandins and Cancer, August 30-September 2, 1981, Washington, DC. Lombardi Cancer Center 75 pp., 1981.

Prostaglandins (PGs) have been implicated in a variety of physiological functions. These include actions on smooth muscle tissues, stimulation of cyclic nucleotides and effects on nonspecific and specific host defense cells in immunity. This study has been initiated for the examination of PG levels in plasma of various cancer patients. The levels of PGs, B, E and F series were examined by radioimmunoassay following extraction and separation on Sephadex LH-20:Silicic acid columns using benzene:ethylacetate:methanol (60:40:5) as solvents. Of 20 active cancer patients significant elevation of the PGE series in plasma was demonstrated except for a breast carcinoma patient. Significant increases in PGs, B and F series in plasma of cancer patients were also shown. Striking changes in the distribution of PGs were especially shown for lung and stomach carcinomas. Increase in PGE series with a decrease in PGF series was demonstrated for the lung cancer group, while a decrease in PGB, and increase in PGE was shown for patients with stomach cancer. It is suggested that serial examination of PG distribution in plasma may be significant in assessing cancer patients. Furthermore, PGs may play a significant role in the regulation of the immune cells associated with the rejection of tumors. (no Refs)

28. IN-VITRO RECONSTITUTION OF BETA-ADRENERGIC RESPONSE IN HUMAN LEUKEMIA CELLS BY CELL-CELL AND MEMBRANE-CELL FUSION (MEETING ABSTRACT).

Schwarzmeier JD, Paietta E, Fischer P
First Medical Clinic, University of Vienna, Lazarettgasse 14, A-1090 Vienna, Austria
Exp Hematol; 9(Suppl9):85 1981

For the first time, Orly and Schramm succeeded in the functional transplantation of hormone receptors from one cell to another. Shortly afterwards, we reported on the complementation of the beta-receptor/adenylate cyclase system not only by fusion of intact cells but also by using membrane fragments as beta-receptor donors. Heterokaryons formed by fusion or adenylate cyclase-deficient S49 mouse lymphoma cells (AC-) and beta-adrenergic receptor-deficient B82 mouse fibroblasts displayed a catecholamine-sensitive adenylate cyclase activity. Comparable results were obtained when B82 fibroblasts were fused with membrane particles from the AC- variant. For standardization of the experimental conditions, human leukemic cell lines grown continuously in culture were chosen. The cell lines were screened for adenylate cyclase activity and superficial beta-adrenergic receptor density. Three types of cell lines expressing differences in the receptor/enzyme system evolved from these studies: Group 1: Cells which exhibited a high number of beta-receptors and a correspondingly strong adenylate cyclase stimulation by isoproterenol, a potent beta-adrenergic agonist. Group 2: Cells which exhibited an equally high beta-receptor density but completely lacked adenylate cyclase response to isoproterenol. Group 3: Cells which neither possessed a remarkable number of beta-receptors nor showed any adenylate cyclase response to isoproterenol, despite a pronounced, response of the enzyme to prostaglandin E1. Cells from groups 2 and 3 were chosen for experiments with the cell-cell and membrane fusion technique. It could be demonstrated that the polyethyleneglycol-induced fusion of beta-receptor deficient cells (group 3) with intact cells or membrane particles from beta-receptor replete cells (group 2) resulted in the reconstitution of the beta-adrenergic response in the fusion products. This response could be specifically blocked by the beta-adrenergic antagonist propranolol. (2 Refs)

29. INHIBITION OF TUMOR CELL ADHERENCE BY PGE1 AND PGI2 (MEETING ABSTRACT).

Fantone J, Kunkel S, Varani J
Department of Pathology, University of Michigan School of Medicine, Ann Arbor, MI, 48109
International Conference on Prostaglandins and Cancer, August 30-September 2, 1981, Washington, DC. Lombardi Cancer Center 75 pp., 1981.

Prostaglandins (PGs) and thromoboxanes are known to be potent mediators of both neutrophil and platelet adhesiveness. Several reports suggest that the potent anti-aggregatory PGs, PGI2 and PGD2, decreased tumor cell metastasis by inhibiting platelet adherence to tumor cells thereby blocking the adherence of the tumor cell-platelet aggregates to endothelium. Recent in vitro studies in our laboratory have shown that Walker Carcinosarcoma Cells (WCC) show increased adherence to plastic plates and bovine corneal endothelium when stimulated with chemotactic agents, N-formyl-methionyl leucyl-phenylalanine and trypsinized fragments of C5, as well as phorbol myristate acetate (PMA), a potent membrane stimulator. When WCC are pre-treated with either PGE1 or PGI2 in the presence of theophylline there is 56.8 +/-4.8% and 50.4 +/-4.2% inhibition of PMA stimulated adherence to plastic plates. Similar effects are observed when WCC are pre-treated with either histamine or dibutyryl cyclic AMP (cAMP). These studies show that the anti-aggregatory PGs, PGE1 and PGI2 can inhibit tumor cell adhesiveness in vitro and suggest that the inhibition of meta stasis may be due to the direct effect of these agents on the tumor cell. These effects may be mediated through alteration of intracellular cAMP levels. (no Refs)

30. TWO HUMAN EXOCRINE PANCREATIC CANCERS ESTABLISHED IN ATHYMIC MICE: SECRETORY ENZYMES, MEMBRANE RECYCLING, AND SOME HORMONE RECEPTORS (MEETING ABSTRACT).

Estival A, Balas D, Clemente F, Ribet A
INSERM U 151 CHU Rangueil, 31400 Toulouse, France
Gastroenterology; 80(5,part2):1143 1981

Two human pancreatic adenocarcinoma (Kc P1, Kc P2) with different growth rates were established as xenographs in nude mice. The existence of normal secretory enzymes in the tumor secretory granules was analyzed using immunocytochemical techniques, with antibodies directed against normal human pancreatic juice. The plasma membrane turnover was studied using cationic ferritin. Endocytic vesicles are quickly labelled and reach the inner golgian zone in the 10th min. Numerous endocytic vesicles containing ferritin were directly fusioning with condensing vacuoles. From the 30th to the 120th min the tracer was also observed in condensing vacuoles and secretory granules. These findings suggest a short pathway and recycling of plasma membrane components probably related with a defect on the first stages of protein synthesis. Some hormone receptors were also analyzed in the two established tumors. Vasoactive intestinal peptide (10-7M) and carbachol (10-6M) respectively increase cyclic (c) AMP and cyclic guanosine monophosphte (cGMP) levels. Secretin stimulates adenylate cyclase activity, the maximal response is reached at high hormone concentrations 10-5M -10-6M. Dopamine increases the cAMP level with a maximal response for 10-5M. Caerulein at concentration as low as 10-11M stimulates guanylate cyclase activity. In conclusion: 1) Normal secretory enzymes were not found. 2) Abnormal membrane recycling is demonstrated. 3) The two human cancers possess carbachol, dopamine, caerulein and secretin receptors; the responses evoked by the two last hormones resemble that of acinar cells more than that of duct cells. (no Refs)

CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES

31. STIMULATION BY PHOSPHATIDIC ACID OF CALCIUM INFLUX AND CYCLIC GMP SYNTHESIS IN NEUROBLASTOMA CELLS.

Ohsako S, Deguchi T
Dept. Biology, Nagoya Univ., Chikusaku, Nagoya, Japan
J Biol Chem; 256(21):10945-10948 1981

Phosphatidic acid added to the medium markedly elevated intracellular cyclic GMP content in cultured neuroblastoma N1E 115 cells. There was a significant elevation of cyclic GMP with 1 ug/ml and a max (70-fold) elevation with 100 ug/ml of phosphatidic acid. Other natural phospholipids did not increase, or increased only slightly the cyclic GMP content in the cells. The elevation of cyclic GMP content by phosphatidic acid was absolutely dependent on extracellular calcium. Phosphatidic acid stimulated the influx of calcium into neuroblastoma cells two- to five-fold. The pattern of the calcium influx induced by phosphatidic acid was comparable to that of cyclic GMP elevation. The stimulation of calcium influx by phosphatidic acid was also observed in cultured heart cells, indicating that phosphatidic acid acts as a calcium ionophore or opens a specific calcium-gate in a variety of cell membranes. Treatment of neuroblastoma cells with phospholipase C increased 32Pi labeling of phosphatidic acid, stimulated the influx of calcium, and elevated the cyclic GMP content in the cells. Thus exogenous as well as endogenous phosphatidic acid stimulates the translocation of calcium across cell membranes and, as a consequence, induces the synthesis of cyclic GMP in the neuroblastoma cells. (Author abstract) (32 Refs)

32. RECOGNIZING HYPERPARATHYROIDISM CAUSED BY ECTOPIC PRODUCTION OF A SUBSTANCE RESEMBLING PARATHYROID HORMONE.

Ernst P, Kaye M
Montreal General Hosp., 1650 Cedar Ave., Montreal, PQ H3G 1A4, Canada
Can Med Assoc J; 125(8):857-859 1981

Criteria for differentiating hyperparathyroidism due to parathyroid adenoma from that due to ectopic secretion of a tumor is presented by way of an illustrative case study. The study involved a 75 yr old man suffering from hypercalcemia secondary to an occult squamous cell carcinoma of the lung. Symptoms included a decrease in well being, anorexia, weakness, 4.5 kg wt loss, chronic cough, increase in the serum concentration of calcium (3.5 milliM mM/l), and creatinine(150uM/l), and decrease in the serum concentration of phosphorus (0.7 mM/l). Urine levels of calcium, cyclic adenosine 3'5'monophosphate(cyclic AMP) and tubular reabsorption were decreased (10.9 mM, 1.7 uM/g creatinine, and 53%, respectively). Measurement of parathyroid hormone(PTH) levels were inconsistent. Other investigative procedures, including chest roentgenography and bone biopsies were interpreted as normal. Focal seizures led to discovery of two brain metastases; a review of the chest roentgenograms and tomography of the mediastinum led to the diagnosis of squamous cell carcinoma of the lung. Distinguishing features of pseudohyperparathyroidism include, most importantly, a short (2-6 mo) history, pronounced wt loss (often greater than 10 kg), renal calculi in less than 10% of cases, absence of pancreatitis and peptic ulcers, and serum calcium levels greater than 3.5 mM/l. Helpful specialized studies include serum concentration of immunoreactive PTH(increased levels suggest primary hyperparathyroidism) and urinary excretion of cyclic AMP(low values suggest pseudohyperparathyroidism). Hypercalcemia is a common clinical problem with diagnosis usually resting between hyperparathyroidism and pseudohyperparathyroidism. (15 Refs)

PROTEIN KINASE STUDIES

33. STUDIES ON THE PHOSPHORYLATION AND SYNTHESIS OF TYPE I REGULATORY SUBUNIT OF CYC LIC AMP-DEPENDENT PROTEIN KINASE IN INTACT S49 MOUSE LYMPHOMA CELLS.

Steinberg RA, Agard DA
Biological Sciences Group, Univ. Connecticut, U-44, Storrs, CT
J Biol Chem; 256(21):11356-11364 1981

Phosphorylation and synthesis of Type I regulatory subunit (RI) of cAMP-dependent protein kinase were studied using two-dimensional polyacrylamide gel electrophoresis of [35S]methionine-labeled proteins from intact S49 mouse lymphoma cells. [32P]Phosphate labeling, peptide mapping, and acid hydrolysis confirm that charge heterogeneity in RI results from phosphorylation of a single serine residue. In drug-free cells, phosphorylation proceeds to a steady state proportion of 90 to 95% of total RI with a half-time of about 25 min. The rate and steady state extent of RI phosphorylation are reduced by some, but not all, agents causing intracellular kinase activation. These results suggest that RI might assume different conformations in association with different amounts of cAMP or different analogs of cAMP. Endogenous kinase activation has no immediate effect on RI synthesis but leads to a moderate increase in RI synthesis after several hours; this induction occurs with all agents tested. Mutants of S49 cells lacking catalytic activity of cAMP-dependent protein kinase exhibit reduced phosphorylation and synthesis of RI. Comparative studies suggest that the phosphorylation of RI and its induction by kinase activation are fairly general phenomena; the extent of RI phosphorylation and the relative rate of RI synthesis are variable among cell types. (Author abstract) (41 Refs)

34. A CELLULAR PROTEIN PHOSPHORYLATED BY THE AVIAN SARCOMA VIRUS TRANSFORMING GENE PRODUCT.

Erikson E, Erikson RL
Health Sciences Center, Univ. Colorado, 4200 E. Ninth Ave., Denver, CO, 80262
Haematol Blood Transfus; 26:397-401 1981

Experiments are described which demonstrate that a newly phosphorylated protein in avian sarcoma virus (ASV)-transformed chicken cells with a mol wt of approx 34,000 (34K) and an isoelectric focusing of about 7.5 is a src-specific substrate found in normal cells and that upon transformation it is phosphorylated in ASV-infected cells of both avian and mammalian origin. This protein appears to be a substrate for the phosphotransferase activity associated with pp60src. This protein, or an analogous protein was partially phosphorylated in all ASV-transformed cells examined to date. The pp60src-specific nature of the phosphorylation is shown by the phosphorylation of 34K by pp60src in vitro at a site identical to that phosphorylated in vitro at a site identical to that phosphorylated in transformed cells. This result strongly suggests that 34K is a direct rather than indirect substrate of pp60src in the cell. This result also suggests that pp60src acts as a protein kinase in the transformed cell as well a s in vitro and that it mediates transformation, at least in part, by phosphorylation of 34K and perhaps of other normal cell proteins. This conclusion does not eliminate the possibility that pp60src may have other functions that influence transformation. (11 Refs)

35. TUMOR PROMOTERS ALTER GENE EXPRESSION AND PROTEIN PHOSPHORYLATION IN AVIAN CELLS IN CULTURE.

Laszlo A, Radke K, Chin S, Bissell MJ
Lab. Cell Biology, Div. Biology and Medicine, Univ. California, Berkeley, CA, 94720
Proc Natl Acad Sci USA; 78(10):6241-6245 1981

We have investigated the effect of 12-O-tetradecanoylphorbol 13-acetate (TPA) on the synthesis and modification of polypeptides in normal avian cells and cells infected by wild-type and temperature-sensitive Rous sarcoma virus (RSV). Using two-dimensional gel electrophoresis, we have detected alterations in both the abundance of cellular polypeptides and in their phosphorylation that seem unique to TPA treatment. However, the state of phosphorylation of the major putative substrate for the action of the src gene-associated protein kinase, the 34-to 36-kilodalton protein, was not altered. Moreover, examination of the phosphorylated amino acid content of total cellular phosphoproteins revealed that the response to TPA was not associated with detectable increases in their phosphotyrosine content. These results make it unlikely that TPA acts by the activation of the phosphorylating activity of the cellular proto-src gene or by the activation of other cellular phosphotyrosine-specific kinases. We have shown previously that temperature-sensitive RSV-infected cells at nonpermissive temperature demonstrate an increased sensitivity to TPA treatment. Our present results indicate that this is not due to reactivation of the phosphorylating activity of the defective src gene product or to its leakiness, and they lend support to the notion of multistep viral carcinogenesis. (Author abstract) (29 Refs)

36. CHARACTERIZATION OF SITES FOR TYROSINE PHOSPHORYLATION IN THE TRANSFORMING PROTEIN OF ROUS SARCOMA VIRUS (PP60V-SRC) AND ITS NORMAL CELLULAR HOMOLOGUE (PP60C-SRC).

Smart JE, Oppermann H, Czerailofsky AP, Purchio AF, Erikson RL, Bishop JM
Cold Spring Harbor Lab., Cold Spring Harbor, NY, 11724
Proc Natl Acad Sci USA; 78(10):6013-6017 1981

The transforming protein of Rous sarcoma virus (pp60v-src) and its normal cellular homologue (pp60c-src) appear to be protein kinases that phosphorylate tyrosine in a variety of protein substrates. In addition, pp60v-src and pp60c-src are themselves phosphorylated on serine and tyrosine. It is likely that these phosphorylations serve to regulate the function(s) of pp60v-src and pp60c-src. We have therefore characterized the sites of tyrosine phosphorylation in the two proteins. Tyrosine phosphorylation of pp60v-src in infected cells occurs mainly (if not entirely) at residue 419 in the deduced amino acid sequence of the protein. Surrounding this residue is the sequence Leu-Ile-Glu-Asp-Asn-Glu-Tyr(P)-Thr-Ala-Arg. This peptide is distinguished by the fact that three out of the four amino acids that precede the phosphorylated tyrosine are acidic in nature. These results define what may prove to be a widely used site for tyrosine phosphorylation in the regulation of cellular function. The same site was phosphorylated when partially purified pp60v-src was used in a phosphotransfer reaction in vitro. The results with pp60c-src were more complex. the site of tyrosine phosphorylation in vitro appeared to be the same as that found in pp60v-src. By contrast, phosphorylation of pp60c-src in vivo apparently occurred at a different, and currently unidentified, tyrosine residue. It is therefore possible that pp60v-src and pp60c-src respond differently to regulatory influences in the intact cell. (Author abstract) (34 Refs)

37. CORRELATED LOSS OF THE TRANSFORMED PHENOTYPE AND PP60SRC-ASSOCIATED PROTEIN KINASE ACTIVITY.

Friis RR, Ziemiecki A, Bosch V, Boschek CB, Bauer H
Institut fur Virologie, Justus-Liebig-Universitat, Frankfurter Strasse 107, 6300 Giessen, W. Germany
Haematol Blood Transfus; 26:402-404 1981

NY68 cells were infected with a transformation-defective, temperature-sensitive mutant of Rous sarcoma virus. The protein kinase activity of the viral gene product pp60src declined rapidly when the infected cells were shifted to 42 C (nonpermissive for virus growth). Loss of kinase activity correlated with the reversion of the transformed cells to a normal phenotype (evidenced by changes in cell microfilaments). New protein synthesis was not required for the reversion. (9 Refs)

38. MODIFIED PROTEIN KINASE ACTIVITY AND ISOZYME DISTRIBUTION IN ADENOCARCINOMA OF THE HUMAN COLON.

Raul F, Launay J-F, Galluser M, Doffoel M, Marescaux J, Grenier JF

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Biomedicine [Express]; 35(3):98-100 1981

Protein kinase activity and histone kinase isozyme distribution have been determined in soluble extracts of adenocarcinoma of the human colon and compared to adjacent normal mucosa. The results show an enhancement in endogenous protein kinase activity and the presence of an additional isozyme (PKI) for histone kinase activity in the tumor tissue. PKI activity exhibited a peculiar behavior in comparison to the isozyme. PKII present in both carcinoma and normal mucosa after dialysis of the soluble extracts. It is suggested that alteration of intracellular regulatory processes involved in PKI activity might be related to the maintenance of the proliferate state in human colon carcinoma. (Author abstract) (16 Refs)

39. HUMAN LEUKEMIC LYMPHOCYTES - BIOCHEMICAL PARAMETERS OF THE ALTERED DIFFERENTIATION STATUS.

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Haematol Blood Transfus; 26:225-228 1981

Altered differentiation of neoplastic cells should relate to alterations in cellular regulation. Two parameters of posttransscriptional protein modification were studied: the protein phosphorylation system as represented by the protein kinase and the ADP ribosylation of nuclear proteins. Comparing normal human blood lymphocytes with chronic lymphocytic leukemia (CLL) lymphocytes marked decrease in various protein kinases was observed, including cyclic AMP-dependent enzymes and its subunits (reduction in CLL subunits to less than 20%). Marked reduction in the extent of protein modification by mono(ADP-ribose)-residues was noticed in CLL cells also, and it was due mainly due to ammonia resistant conjugates (normal approx 250-700, CLL approx 25-200). This last reduction was not due to chemotherapy, because it was present in patients receiving chemotherapy and in those without. Besides effective chemotherapy treatment was accompanied with decrease in leukocytes and increasing levels of ADP-ribose residues. CLL cells represent G0 type tumor cell and alterations in nuclear mono(ADP)-ribosylation appear to be characteristic of tumors independently of their growth rate. Quantitation of ammonia resistant protein conjugates could be developped as a general test for the determination of the degree or of the tumor cell differentiation. (9 Refs)

40. EPIDERMAL GROWTH FACTOR: I. MORPHOLOGICAL EFFECTS ON HUMAN CARCINOMA CELLS. II. RELATEDNESS OF THE EGF RECEPTOR-KINASE TO A VIRAL TRANSFORMING PROTEIN.

Chinkers MD
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Diss Abstr Int [B]; 42(4):1272-B 1981

This dissertation is divided into two parts, each concerned with the interaction of epidermal growth factor (EFG) with A-431 human epidermoid carcinoma cells. EGF induces rapid and dramatic shape changes in these cells; the exact nature of these changes depends on whether Ca++ is present in the medium. After several hr, as cells retracting at the edges of colonies pile up on top of each other, multilayered colonies are formed. This multilayered morphology persists for several days in the presence of EGF, but is freely reversed upon removal of EGF from the medium. The second part of the dissertation concerns the interaction of affinity-purified EGF receptor, prepared from A-431 cells, with antibodies to the transforming protein, pp60src, of Rous sarcoma virus (RSV). This study was initiated, in part, because (a) the EGF-induced morphological changes described above are similar to RSV-induced cell shape changes and (b) both pp60src and the partially purified EGF receptor possess tyrosine-specific protein kinase activity, the latter being stimulated by EFG. The EGF-stimulated kinase, like pp60src, specifically phosphorylates the heavy chain of anti-pp60src IgG; this phosphorylation occurs on tryosine and is stimulated by EGF. However, neither the receptor nor its associated kinase activity are precipitated by these antisera, even those able to precipitate the normal cellular homologue of pp60src (pp60sarc). These results suggest that while the EGF-stimulated kinase is probably not pp60sarc, it may be related to that enzyme. (no Refs)

41. TURNOVER OF REGULATORY SUBUNIT OF CYCLIC AMP-DEPENDENT PROTEIN KINASE IN S49 MOUSE LYMPHOMA CELLS.

Steinberg RA, Agard DA
Biological Sciences Group, U-44, Univ. Connecticut, Storrs, CT, 06268
J Biol Chem; 256(21):10731-10734 1981

Turnover of regulatory subunit (R) of Type I cAMP-dependent protein kinase in intact S49 mouse lymphoma cells was studied using two-dimensional gel electrophoresis to analyze [35S]methionine label in R during label-chase experiments. R decays exponentially with a half-life of about 8.4 hr in drug-free, wild type cells. In mutant cells lacking functional kinase catalytic subunit, R is about 10x more labile than in wild type cells. 8-bromo-cAMP, isoproterenol, and cholera toxin destabilize R in wild type cells to an extent comparable to the 'kinase-negative' mutation. In contrast, dibutyryl-cAMP stabilizes R in both wild type and kinase-negative cells. Sodium butyrate has no significant effect on R stability. These results are discussed in terms of R structure and the regulation of R expression. (Author abstract) (25 Refs)

42. ACTIVATION OF PURIFIED HEPATOMA RNA POLYMERASE I BY HOMOLOGOUS PROTEIN KINASE NII.

Duceman BW, Rose KM, Jacob ST
Dept. Human Genetics, Yale Univ. Coll. Medicine, New Haven, CT, 06510
J Biol Chem; 256(21):10755-10758 1981

We have recently purified a cyclic nucleotide-independent, heparin-sensitive nuclear protein kinase (NII) from Morris hepatoma 3924A and demonstrated an apparent relationship of this kinase to the two subunits (Mr = 42,000 and 24,600) of RNA polymerase I. When homogeneous protein kinase NII was recombined with purified homologous RNA polymerase I containing limiting quantities of endogenous kinase, RNA synthesis was stimulated as much as five-fold during a 90-min incubation. The enhanced RNA synthesis was due to an increase in the av RNA chain length; protein kinase did not alter the number of RNA molecules synthesized by the polymerase. Phosphorylation of RNA polymerase occurred at serine and threonine moieties. Unlike the NII kinase, purified homologous NI kinase did not phosphorylate RNA polymerase I and, as a result, did not alter transcription. These data indicate that (1) RNA polymerase I is activated by protein kinase NII, (2) endogenous protein kinase NII remaining with highly purified RNA polymerase I does not fully phosphorylate RNA polymerase I in vitro, and (3) protein kinase NII is capable of regulating RNA polymerase I activity by preventing premature termination of RNA chains. (Author abstract) (21 Refs)

43. RELATIONSHIP OF THE FELINE ONCORNAVIRUS ASSOCIATED CELL MEMBRANE ANTIGEN TO A FELINE SARCOMA VIRUS ENCODED POLYPROTEIN.

Snyder HW, Dutta-Choudhury M, Hardy Jr WD
No affiliation given
Haematol Blood Transfus; 26:488-491 1981

Using immunoaffinity chromatography on a column of Sepharose containing bound IgG from cat anti-feline oncornavirus-associated cell membrane antigen (FOCMA) serum, a 70,000-dalton protein was isolated from lysates of mink cells transformed by the Gardner-Arnstein strain of feline sarcoma virus (GA-FeSV). This result was unanticipated because previous evidence had suggested that FOCMA was associated with the much larger (95,000 dalton) gag-x fusion protein in these cells. The GA-FeSV gag-x protein was also isolated by virtue of its binding to anti-FeSV antibodies on a different affinity column. Preliminary biochemical characterization of the 70,000-dalton protein from both GA-FeSV-transformed mink fibroblasts and feline lymphosarcoma cells showed no differences between them. The gag-x protein was distinguishable by labelling with 32P-orthophosphate, association with protein kinase activity, and its precipitation ability with antibodies in a hyperimmune antiserum to FeLV structural proteins. It is hypothesized that FOCMA may actually be a family of antigens, not all of which cross-react with FeSV polyprotein antigens. (21 Refs)

44. PROTEIN KINASES AND GROWTH CONTROL IN HUMAN MAMMARY CARCINOMA CELLS (MCF-7) (MEETING ABSTRACT).

Kung W, Handschin J, Roos W, Eppenberger U
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Experientia; 37(6):651 1981

The human mammary carcinoma cell line MCF-7 is used as a model for hormone-sensitive tumor proliferation. It was suggested that cyclic AMP, via protein kinases, is involved in growth control. However, we show that DBcAMP and theophylline have no effects on proliferation of MCF-7 cells. Analysis of protein kinase activity with diethylaminoethanol cellulose chromatography showed 2 cyclic AMP-dependent and at least 2 cyclic nucleotide-independent protein kinases. The activities of these enzymes were studied under different growth conditions. Growth arrest caused by serum depletion correlates with a marked decrease of a cyclic nucleotide-independent protein kinase eluting at approx 90 mM NaCl. The activities of the cyclic (c) AMP-dependent protein kinases were not substantially affected. In MCF-7 cells, the role of cAMP in growth control needs therefore further critical evaluation. (no Refs)

45. LYMPHOID CELL TRANSFORMATION BY ABELSON MURINE LEUKEMIA VIRUS (MEETING ABSTRACT).

Baltimore D
Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, 02139
Thirteenth Miami Winter Symposium: Cellular Responses to Molecular Modulators, January 12-16, 1981, Miami, Florida. University of Miami 112 pp., 1981.

When bone marrow or fetal liver cells are exposed in culture to Abelson murine leukemia virus (A-MulV) the virus induces the formation of colonies of immortalized lymphoid cells. The phenotype of these cells is variable but the established protein markers in the cell indicate that they are related to pre-B-lymphoid cells. The virus is able to transform these cells by virtue of a protein synthesized under the direction of the virus. The viral RNA is evolved partly from a normal cellular gene and the protein formed by the virus is a hybrid of virus-specific and cell-specific polypeptide sequence. This polypeptide has associated with it protein kinase activity that forms a phosphotyrosine bond between the A-MuLV protein itself and the gamma-phosphate derived from ATP. We have analyzed the structure of the normal cell gene corresponding to the A-MuLV cellular insert and know that it has a specific chromosomal location and a structure including a number of exons and introns. A large segment of the normal cell gene has been cloned and is under detailed investigation. Properties of the A-MuLV protein suggest that it is related to pathways used in the cell to respond to hormonal stimulations. (3 Refs)

OTHER RELATED STUDIES

46. MEMBRANE ALTERATIONS IN FUSING AND PHORBOL ESTER-TREATED MYOGENIC CELLS.

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University of South Florida, Tampa, FL, 33620
Diss Abstr Int [B]; 42(4):1431-B 1981

Cultured chick embryo myogenic cells differentiate and fuse to form multinucleated myotubes which become striated and acquire the potential to undergo Spontaneous Contractions. The diterpene tumor promoter, phorbol-12-myristate 13-acetate (PMA) inhibits myoblast fusion. The most rapid effects of this lipophilic compound occur in plasma membrane-associated processes, such as ion transport and cyclic nucleotide generation. Changes in specific membrane proteins have also been reported to occur in PMA-treated myogenic cells. In addition to the reported alterations in the levels of membrane proteins of known function it was possible that PMA extensively altered the composition and metabolism of other plasma membrane proteins whose functions are unknown. This hypothesis was tested using radiolabeled leucine and mannosamine precursors and electrophoretic analysis of membrane proteins on polyacrylamide gels. By 8 hr incubation PMA altered plasma membrane protein and sialoglycoprotein composition. Stimulation of diacylglycerol levels (2-fold) and stimulation of the rate of label incorporated into phosphatidic acid (2-fold), phosphatidylinositol (3-fold) and phosphatidylcholine (2-fold) were observed within 15-30 min of treatment with PMA. The increase in each of these lipids was shown to arise by synthesis from pre-existing lipids rather than by de novo synthesis. Using a double-label turnover technique and fatty acid analysis, it was shown that phosphatidylcholine is the most probable precursor of the PMA-induced diacylglycerol fraction. Subcellular fractionation of the PMA-stimulated cells suggested that the diacylglycerol was generated in the plasma membrane. Possible models for the mechanisms of PMA-dependent alterations in lipid metabolism and myoblast fusion are discussed. (no Refs)

47. CHARACTERIZATION OF CALCITONIN- AND ADRENOCORTICOTROPIN-PRODUCING HUMAN CLONED CELL LINES.

Dermody WC, Rosen MA, Ananthaswamy R, Levy AG, Hixson CV, Aldenderfer PH, Nelson-Rees WA, Marangos PJ, Swanson EC
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J Clin Endocrinol Metab; 53(5):970-977 1981

We have developed three human cloned cell lines that produce immunoreactive human calcitonin (ihCT) and ACTH (iACTH) as well as exhibit characteristics of cultured neural cells. Clones HMS-41/I,-78/2, and-98/2 were developed from cell lines HeLa AV3, MBA 9812 (bronchogenic carcinoma), and SW 267 (pheochromocytoma), respectively. Karyological analysis of both the parent and the cloned cell lines confirmed the identity of HeLa AV3 and MBA 9812. When grown in serum-free media designed for culturing neural cells, the patterns of production for both ihCT and iACTH varied among the clones. The multiple patterns of hormone production suggest that the mechanisms involved in the biosynthesis, processing, and secretion of these hormones differ among the clones. The clones contain neuron-specific enolase and the putative neurotransmitters beta-alanine and gamma-amino butyric acid, and they respond to cAMP analogs by differentiating, as noted by the extension of neurites (except the HeLa-derived HMS-41/I). The iACTH extracted from cells and synthetic ACTH exhibited equivalent profiles upon isoelectric focusing. The forms of ihCT noted in cell extracts were similar to those observed in extracts of human tumor tissue. Our rabbit antiserum to hCT failed to detect ihCT in those cell extracts prepared for ACTH determination or in extracts of rat pituitaries, but it did detect CT in rat thyroids by both RIA and immunofluorescent procedures. We concluded that our antisera to hCT do not detect the precursor form of ACTH. The availability of these cloned cell lines provides model systems for examining the production of these peptide hormones and the concomitant expression of neural and endocrine characteristics. (Author abstract) (56 Refs)

48. NEURON-SPECIFIC ENOLASE IN RELATION TO DIFFERENTIATION IN HUMAN NEUROBLASTOMA.

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Brain Res; 224(1):69-82 1981

Biopsy material was analyzed for neuron-specific enolase (NSE) after separation with ion exchange chromatography. Biopsy cells from human neuroblastoma were shown to have a much higher NSE content than cells of three cultured human neuroblastoma cell lines. The neuroblastoma cells had lower levels than biopsy cells of ganglioneuroblastoma, a more differentiated neuroblastoma. After treatment of cultured neuroblastoma cells with nerve growth factor or dibutyryl-cAMP, some cells showed morphologic differentiation and simultaneous increase in NSE levels. Results indicate that NSE may prove to be a useful marker for differentiation in human neuroblastoma. (25 Refs)

49. A MET-ENKEPHALIN ANALOG INHIBITS ADRENOCORTICOTROPIN SECRETION BY CULTURED PITUITARY CELLS FROM A PATIENT WITH NELSON'S SYNDROME.

Lamberts SW, Oosterom R, Verleun T, Bons EG, Uitterlinden P
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J Clin Endocrinol Metab; 53(5):1084-1086 1981

ACTH secretion by cultured nonenzymatically dispersed pituitary tumor cells from a patient with Nelson's syndrome was studied. Hormone release was suppressed by 74 +-6% by the addition of 1 uM of the met-enkephalin analog FK 33824, while naloxone (1 uM) stimulated ACTH release by 70 +-5%. Somatostatin, dexamethasone. bromocriptine and cyproheptadine in a concentration of 1 uM each inhibited ACTH release by 25 +-2%, 35 +-2%, 52 +-2%, and 61 +-4%, respectively, while lysine vasopressin (0.1 uM) and dibutyryl cAMP (5 mM) stimulated ACTH release by 112 +-8% and 220 +-4%, respectively. In conclusion, it was shown that the stimuli mentioned above directly affect ACTH secretion by the pituitary tumor cells. The inhibitory action of the met-enkephalin analog and the stimulatory action of naloxone on ACTH secretion make the presence of opiate receptors on this type of tumor likely. (Author abstract) (15 Refs)

50. PRESENCE OF ECTOPIC BETA-ADRENERGIC RECEPTORS ON HUMAN ADRENOCORTICAL CORTISOL-PRODUCING ADENOMAS.

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J Clin Endocrinol Metab; 53(5):953-957 1981

A direct binding study of radioligand [3H]dihydroalprenolol (DHA), a potent beta-adrenergic antagonist, was performed on the particulate fractions of four adrenocortical adenomas (3 cortisol-producing adenomas and 1 aldosterone-producing adenoma) and normal adrenal tissues. The effect of epinephrine on cortisol production was also evaluated in vitro from the cultured tumor cells from one cortisol-producing adenoma. Saturable binding of [3H]DHA to the tumor membranes was observed in two of three cortisol-producing adenomas, but not in the aldosterone-producing adenoma or in normal adrenal tissues. Scatchard analysis of equilibrium binding of [3H]DHA revealed a single class of binding sites on the tumor membranes; the apparent dissociation constant (Kd) was 1 nM in each, and the numbers of binding sites were 108 and 45 fmol/mg protein, respectively. Competition by adrenergic agents with [3H]DHA for binding sites on the membranes from one cortisol-producing adenoma revealed that (+-)propranolol, a beta-adrenergic antagonist, was about 350-fold more potent than phentolamine, an alpha-adrenergic antagonist, suggesting the beta-adrenergic nature of receptor sites. In addition, stereospecificity was demonstrated by about 1,000-fold greater affinity of (-)alprenolol than to (+)alprenolol, both of which are stereoisomers of the beta-adrenergic antagonist. Furthermore, production of cortisol from the cultured tumor cells prepared from the same adenoma was significantly stimulated by epinephrine in addition to ACTH. These data indicate that ectopic beta-adrenergic receptor sites are present in some human adrenocortical tumors which may be functionally related to the activation of adenylate cyclase by catecholamines other than ACTH in those tumors, as previously demonstrated. The mechanism by which such altered cellular membrane characteristics occur in association with neoplastic alteration of the endocrine tissues remains unanswered. (Author abstract) (20 Refs)

51. MALIGNANCY-ASSOCIATED HYPERCALCEMIA: EVALUATION WITH A CYTOCHEMICAL BIOASSAY FOR PARATHYROID HORMONE.

Goltzman D, Stewart AF, Broadus AE
Dept. Medicine, Royal Victoria Hosp., Montreal H3A 1A1, Canada
J Clin Endocrinol Metab; 53(5):899-904 1981

Employing a cytochemical assay initially developed for measuring parathyroid hormone (PTH), bioactivity was assessed in 33 patients with malignancies. Initial studies in vitro were consistent with a role for cAMP as a second messenger in the bioassay. Cytochemical bioactivity was increased in the peripheral plasma of 10 of 16 hypercalcemic patients with elevated nephrogenous cAMP excretion, and mean levels were 10-fold higher in these patients than in 17 normocalcemic or hypercalcemic patients with normal or suppressed nephrogenous cAMP excretion, respectively. Plasma bioactivity, serum calcium, and nephrogenous cAMP excretion all fell to normal in one patient after tumor resection, and cytochemical bioactivity was demonstrable in the tissue culture medium in which the neoplasm was maintained. Gel chromatographic analysis revealed that a major component of plasma bioactivity eluted before rather than with PTH-(1-84) in patients with malignancy in contrast with that in patients with primary hyperparathyroidism. The studies, therefore, demonstrate the capacity of the cytochemical bioassay to measure increased activity in patients with malignancy, hypercalcemia, and elevated nephrogenous cAMP excretion; suggest that the material responsible for the activity differs from PTH-(1-84); and provide a sensitive detector system for further analysis of this material and its role in the pathogenesis of this disease. (Author abstract) (22 Refs)

52. BIOCHEMICAL PARAMETERS IN HEAD AND NECK CANCER PATIENTS WITH HYPERCALCEMIA (MEETING ABSTRACT).

Angel MF, Stewart A, Pensak ML, Pillsbury HR, Sasaki CT
No affiliation given
Otolaryngol Head Neck Surg; 89(5,section2):198 1981

Hypercalcemia associated with head and neck malignancy is a common occurrence; its causes are multiple. Eight hypercalcemic patients with head and neck malignancy were studied. Nephrogenous cyclic AMP, tubular phosphorus threshold, fasting calcium excretion, plasma 1,25 dihydroxyvitamin D, and immunoreactive parathyroid hormone were measured. Excessive dietary calcium administration was the cause of hypercalcemia in two patients. Six patients demonstrated humorally mediated hypercalcemia. These patients were similar to patients with primary hyperparathyroidism in that both had elevated nephrogenous cyclic AMP excretion and reduced proximal tubular phosphorus reabsorption. They differed from patients with primary hyperparathyroidism by having normal levels of immunoreactive parathyroid hormone, sharply increased fasting calcium excretion, and strikingly reduced mean plasma levels of 1,25 dihydroxyvitamin D. These data strongly suggest that the humoral factor responsible for hypercalcemia in patients with head and neck cancer is not parathyroid hormone, but a parathyroid-like hormone with unique biochemical actions. (no Refs)

53. SPECIFIC BINDING OF PHORBOL ESTERS BY PANCREATIC ACINI AND ACINAR CARCINOMA CELLS (MEETING ABSTRACT).

Gunther GR
St. Jude Children's Research Hospital, Memphis, TN
Newsletter Natl Pancreas Cancer Project; VI(2):24 1981

The phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA) triggers the discharge of secretory protein from pancreatic acini in an energy-dependent manner, without stimulating Ca + + efflux or elevating intracellular levels of cyclic nucleotides. Since receptors for TPA have been identified on other cell types, a search for specific binding to guinea pig pancreatic acini was carried out using the radiolabeled phorbol derivative (20-3H)-phorbol-12,13-dibutyrate (3H-PDBu). PDBu also stimulates discharge, with an optimally stimulating dose (3x10(-6) M) 10-fold higher than that required for TPA. 3H-PDBu binding was found to be specific in that up to 70% of the cell-associated radioactivity was inhibitable by excess unlabeled PDBu. Specific binding reached a max in either 5 min at 20 C or 2 hr at 4 C, and Scatchard analysis under the two conditions gave equivalent results. PDBu bound to 8.0 x 10(4) sites/pgm DNA with a KD of 1.1 x 10(-7) M. The PDBu binding sites are apparently of two types, however, since TPA and other phorbol esters with secretagogue activity compete for only 30-40% of the available PDBu receptors. Phorbol derivatives that do not stimulate discharge do not inhibit 3H-PDBu binding, and binding is not affected by caerulein, carbamylcholine, secretin or vasoactive intestinal peptide. Phorbol ester receptors with similar characteristics were also found on normal rat acini and on cells from a rat acinar carcinoma. These findings are consistent with the hypothesis that phorbol esters trigger pancreatic discharge through interaction with specific cellular receptors. (no Refs)

54. MULTICELLULAR TUMOUR SPHEROIDS FROM HUMAN BRAIN TUMORS. A MODEL FOR EXPERIMENTAL CHEMOTHERAPY IN VITRO? (MEETING ABSTRACT).

Oktar N, Darling JL, Thomas DG
Department of Neurological Surgery, Institute of Neurology, Queen Square, London WC1N 3BG, England
Cell Biol Int Rep; 5(SupplA):42 1981

Multicellular tumor spheroids have been obtained from a range of fourteen primary and secondary human brain tumors. Tumor biopsies were minced, disaggregated with collagenase and the resultant cell suspension diluted to 5x10(5) cell/ml and inoculated into flasks coated with 0.5% agar. After approx one wk in static culture, single spheroids (mean vol 1.4 x 10(6) um(3)) were transferred into individual agar coated wells on a microtitration plate. At least 10 spheroids were used for each trial. They were exposed to graded concentrations of various chemotherapeutic agents (lomustine, Procarbazine, Vincristine, VM-26, Adriamycin, Mithramycin, L-asparaginase and Methotrexate) for 24 hr at 37 C. After treatment, drugs were removed, the spheroids washed and refed with fresh growth medium. The growth medium of treated and non-treated (control) spheroids was changed twice weekly throughout the assay. The spheroids were measured daily using an eye piece um for a 10 day period following drug removal and the growth delay calculated. Spheroids were also disaggregated after drug treatment and the colony forming efficiency in soft agar was determined. A dose dependant correlation was observed between both relative growth delay and cell survival with these cytotoxic agents. However, patterns of chemotherapeutic response were found to vary considerably between different tumor types and even between tumors of similar histopathology. The effects of corticosteroids, interferon and cyclic nucleotides on human tumor multicellular spheroids was also assessed using the same system. Spheroids produced from human brain tumors may be useful as models for the study of general biology as well as a model for experimental in vitro chemosensitivity. (no Refs)

55. BIOCHEMICAL BASIS OF ACTION OF PHORBOL ESTERS ON PANCREATIC ACINAR CELLS: A NEW MECHANISM FOR STIMULATING SECRETION (MEETING ABSTRACT).

Jensen RT, Lemp GF, Gardner JD
National Institutes of Health, Bethesda, MD, 20205
Gastroenterology; 80(5,part2):1184 1981

TPA (12-O-tetradecanoyl-phorbol-13-acetate) is a potent tumor-promoting agent extracted from croton oil. In the present studies we examined the biochemical basis of action of TPA on dispersed acini from guinea pig pancreas. TPA caused a 20-fold increase in amylase secretion and was 70 percent more efficacious than a maximally effective concentration of cholecystokinin (CCK). Detectable stimulation of amylase secretion occurred with 3nM TPA, half-maximal stimulation with 0.1 uM and maximal with 30uM. The action of TPA on enzyme secretion was not altered by secretagogues that cause mobilization of cellular calcium (CCK, carbachol, bombesin, A23187) but was potentiated by secretagogues that increase cellular cyclic AMP (vasoactive intestinal peptide, secretin). TPA, however, did stimulate calcium uptake by pancreatic acini and the dose-response curve for stimulation of calcium uptake was the same as that for stimulation of enzyme secretion. These results indicate that the actions, of TPA on pancreatic acini are unique in two respects: 1) TPA is the most effective pancreatic secretagogue examined to date; 2) although the action of TPA is calcium-mediated, TPA acts by increasing the inward movement of calcium into acinar cells, not by causing mobilization of intracellular calcium. Furthermore, the close correlation between the relative abilities of phorbol esters to stimulate pancreatic enzyme secretion and the abilities of these agents to promote carcinogenesis suggests that these two effects may be caused by a common calcium-mediated mechanism. (no Refs)

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AUTHOR INDEX WITH CITATION NUMBERS

Agard DA 33
Agard DA 41
Aldenderfer PH 47
Ambrus CM 1
Ambrus CM 25
Ambrus JL 1
Ambrus JL 25
Ananthaswamy R 47
Andreis PG 15
Angel MF 52
Armato U 15
Ascoli M 20
Attallah A 25
Balansky R 13
Balas D 30
Baltimore D 45
Bamonti-Catena 2
Bauer H 37
Berger H 8
Bishop JM 36
Bissell MJ 35
Blagoeva P 13
Blakely WF 5
Bons EG 49
Bosch V 37
Boschek CB 37
Braceland BM 3
Breitman TR 11
Broadus AE 51
Butman BT 18
Chapman LF 7
Chin S 35
Chinkers MD 40
Clemente F 22
Clemente F 24
Clemente F 30
Cole RD 4
Cripps C 27
Czernilofsky AP 36
Darling JL 54
De Gaetano G 17
Deguchi T 31
Dermody WC 47
Doffoel M 38
Duceman BW 42
Dunn JR 26
Dutta-Choudhury 43
Eppenberger U 12
Eppenberger U 44
Erikson E 34
Erikson RL 34
Erikson RL 36
Eriksson S 14
Ernst P 32
Estival A 22
Estival A 24
Estival A 30
Fantone J 29
Fischer P 28
Freeman DA 20
Friis RR 37
Fujita T 50
Galluser M 38
Garbrecht M 39
Gardner JD 55
Goltzman D 51
Grenier JF 38
Grotte G 48
Grove RI 46
Grunicke H 8
Gunther GR 53
Handloser K 12
Handschin J 12
Handschin J 44
Hardy Jr WD 43
Harvey PC 27
Hilz H 39
Hirata Y 50
Hixson CV 47
Hjerten S 48
Hokama Y 27
Hollande E 24
Honn KV 26
Honn KV 9
Jacob ST 42
Jensen RT 55
Jentsch J 8
Jin Y 16
Johansson KE 48
Karakousis C 1
Karakousis C 25
Kaye M 32
Keene BR 11
Kemp RG 10
Kimura LH 27
Krishnaraj R 10
Kung W 12
Kung W 44
Kunkel S 29
Lackgren G 48
Lamberts SW 49
Lanks KW 6
Larsson E 48
Laszlo A 35
Launay J-F 38
Lee JB 25
Lemp GF 55
Levy AG 47
Lombardi L 2
Lombardo JM 6
MacManus JP 3
Maiolo AT 2
Mantero F 15
Marangos PJ 47
Marescaux J 38
Martin DW 14
Matsukura S 50
Meyer J 26
Meyer J 9
Morris HP 3
Nelson-Rees WA 47
Nilsson K 48
Odelstad L 48
Ohsako S 31
Oktar N 54
Olsson I 11
Oosterom R 49
Oppermann H 36
Pahlman S 48
Paietta E 28
Pehrson JR 4
Pensak ML 52
Peracchi M 2
Pillsbury HR 52
Polli EE 2
Purchio AF 36
Puschendorf B 8
Radke K 35
Raul F 38
Ribet A 22
Ribet A 24
Ribet A 30
Rixon RH 3
Roos W 44
Rose KM 42
Rosen MA 47
Sasaki CT 52
Schimmer BP 21
Schwabe U 23
Schwarzmeier JD 28
Schwoch G 39
Seyedin SM 4
Smart JE 36
Snyder HW 43
Stefanovich V 1
Stefanovitch V 25
Steinberg RA 33
Steinberg RA 41
Stewart A 52
Stewart AF 51
Sueoka S 50
Swanson EC 47
Takita H 1
Thomas DG 54
Trocheris V 24
Uchihashi M 50
Uitterlinden P 49
Varani J 29
Verleun T 49
Warren JR 19
Watt VM 21
Whitfield JF 3
Wielckens K 39
Ziemiecki A 37

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In this issue:

- **REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES** 1-5
- **REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES** 6-8
- **CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES** 9-14
- **CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS** 15-16
- **CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES** 17-19
- **PROTEIN KINASE STUDIES** 20-25

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service • National Institutes of Health • National Cancer Institute

HE 20. 3173/2:

REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES

1. IMMEDIATE VERSUS POSTPONED COMBINATION CHEMOTHERAPY (CAMP) FOR UNRESECTABLE NON-SMALL CELL LUNG CANCER: A RANDOMIZED TRIAL.

Lad TE, Nelson RB, Diekamp U, Kukla LJ, Sarma PR, Larson CS, Currie ET, Chawla MS, Tichler T, Zawila P, McGuire WP

Dept. Medicine, Univ. Illinois Hosp., PO Box 6998, Chicago, IL, 60680

Cancer Treat Rep; 65(11/12):973-978 1981

A randomized control trial was performed in good performance status patients with unresectable non-small cell lung cancer to test a strategy of early aggressive combination chemotherapy (CAMP [cyclophosphamide, doxorubicin, methotrexate, and procarbazine]) versus a strategy of delaying such treatment until clinical deterioration. Thirty-seven patients received immediate CAMP and 35 patients received initial low-dose single-agent CCNU (CAMP was postponed). Immediate CAMP therapy produced an objective response rate of 44% in patients with measurable lesions, and CCNU produced none. Median survival was 193 days for the immediate-CAMP group and 175 days for the postponed-CAMP group (p = 0.26). Measures of quality of life were made and no difference emerged between the two treatment strategies. This trial failed to show substantial benefit from immediate combination chemotherapy in minimally symptomatic patients with non-small cell lung cancer. (Author abstract) (14 Refs)

2. CHEMOTHERAPY FOR METASTATIC NON-SMALL CELL BRONCHOGENIC CARCINOMA: EST 2575, GENERATION III, HAM VERSUS CAMP.

Ruckdeschel JC, Mehta CR, Salazar OM, Creech RH, Sponzo RW

Div. Oncology, Dept. Medicine, Albany Medical Coll. of Union Univ., Albany, NY, 12208

Cancer Treat Rep; 65(11/12):959-963 1981

Between September 1978 and March 1979 the Eastern Cooperative Oncology Group compared the CAMP (cyclophosphamide, doxorubicin, methotrexate, and procarbazine) and HAM (hexamethylmelamine, doxorubicin, and methotrexate) regimens in 154 patients with metastatic non-small cell bronchogenic carcinoma. Most patients were ambulatory (77%) and had not received prior radiotherapy (59%). HAM produced two complete responses (CR) and eight partial responses (PR) (n = 77; 2.5% CR, 10.4% PR, 13% overall response) whereas CAMP resulted in 5 CR and 12 PR (n = 77; 6.5% CR, 15.6% PR, 22% overall response) This difference was not statistically significant (p = 0.14 with Fisher's exact two-sided test) nor was the difference in overall median survival (HAM, 22.1 wk and CAMP, 19.3 wk). Responders had a significantly improved median survival (32.5 wk) compared to nonresponders (17.9 wk, p = 0.018). Ambulatory performance status and lack of prior radiotherapy were positive predictors for prolonged survival. (Author abstract) (6 Refs)

3. ALTERATIONS IN GROWTH REQUIREMENTS OF KIDNEY EPITHELIAL CELLS IN DEFINED MEDIUM ASSOCIATED WITH MALIGNANT TRANSFORMATION.

Taub M, U B, Chuman L, Rindler MJ, Saier MH, Sato G

Biochemistry Dept., State Univ. New York at Buffalo, Buffalo, NY, 14214

J Supramol Struct Cell Biochem; 15(1):63-72 1981

Association of changes in growth requirements of kidney epithelial cells with malignant transformation was studied. Madin Darby Canine Kidney (MDKC) and baby mouse kidney (BMK) cell lines were cultured on Medium K-1 (serum-free medium supplemented with insulin, transferrin, prostaglandin E1 (PGE1), triiodothyronine (T3), and hydrocortisone). Both cell types grew on K-1 and responded to the individual supplements. Deletion of any single supplement resulted in decreased culture growth. The fewest MDKC or BMK colonies were found if transferrin or PGE1 was deleted. Results indicated MDKC as a suitable model for normal kidney cells in vitro. Malignant transformation of MDKC by Moloney sarcoma virus resulted in reduction of the optimal dose of insulin and the elimination in some cells of the PGE1 requirement. Transformed cells required fibronectin for growth in K-1. Variant cells were tumorigenic in adult nude mice and had altered cyclic AMP metabolism. (20 Refs)

4. CYCLIC ADENOSINE MONOPHOSPHATE CONTENT OF ADIPOSE TISSUE IN BREAST AND LUNG CANCER PATIENTS.

Bershtein LM, Seleznev IK, Lemekhov VG

Lab. Endocrinology, N. N. Petrov Res. Inst. Oncology, Leningrad, USSR

Vopr Med Khim; 27(5):607-611 1981

Cyclic AMP (cAMP) contents of adipose tissues in 33 women with breast cancer (0.284 picomoles/mg) were lower than those in 12 control women (0.313 picomoles/mg), while the cAMP contents of the adipose tissues in 27 men with lung cancer (0.289 picomoles/mg) exceeded slightly those in 15 control men (0.273 picomoles/mg). A negative correlation between cAMP content and adipocyte size was observed in the controls; however, this correlation was absent in both the breast and the lung cancer patients. (21 Refs) (In RUS)

5. EFFECT OF ACTIVATORS OF CAMP ACCUMULATION ON DIFFERENT STAGES OF GENOME EXPRESSION DURING ACUTE RADIATION INJURY. COMMUNICATION 2. EFFECT OF SEROTONIN AND PAPAVERINE ON TEMPLATE ACTIVITY OF NUCLEAR CHROMATIN FROM LIVERS AND SPLEENS OF RATS SUBJECTED TO WHOLE-BODY X-IRRADIATION.

Tsudzevich BA, Galkina LA, Kucherenko NE

Faculty Biology, T. G. Shevchenko State Univ., Kiev, USSR

Radiobiologiia; 21(5):654-659 1981

Administration of serotonin (ST) and papaverine (PV) to rats prior to whole-body x-irradiation prevented the inhibition of template activity of spleen chromatin and stimulated the template activity of liver chromatin. It was suggested that the radioprotective effect of activators of cyclic AMP is associated with increased radioresistance of early stages of genome expression. (11 Refs) (In RUS)

REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES

6. CELL JUNCTION AND CYCLIC AMP: III. PROMOTION OF JUNCTIONAL MEMBRANE PERMEABILITY AND JUNCTIONAL MEMBRANE PARTICLES IN A JUNCTION-DEFICIENT CELL TYPE.

Azarnia R, Dahl G, Loewenstein WR
Dept. Physiology and Biophysics, Univ. Miami, Sch. Medicine, Miami, FL, 33101
J Membrane Biol; 63(1/2):133-146 1981

The effect of cyclic nucleotides on promotion of junctional membrane permeability and differentiation was studied in C1-1D, a strain of murine cancer cells lacking permeable junctions in confluent culture (CC). CC were exposed to 1 mM concentrations of cyclic AMP (cAMP), dibutyryl cAMP (db-cAMP), db-cAMP and caffeine, or cholera toxin. In each case permeable intercellular junctions were formed, and cells transferred carboxyfluorescein to each other. The effects were complete within hours. Similar effects were produced in non-CC and in cultures that were starved for serum, a condition the caused increased cAMP levels. In non-CC, formation of junctions and differentiation of junction membranes was inversely proportional to cell density. Cells in low-density cultures were able to form junctions with human Lesch-Nyhan or murine 3T3-Balb C cells. Electrical coupling of cells was not inhibited by the cytokinesis inhibitor cytochalasin B (1 ug/ml). The protein synthesis inhibitor cycloheximide prevented formation of recognizable gap junctions and tight junctions in CC treated with db-cAMP. (37 Refs)

7. EXPRESSION OF GLIAL AND VIMENTIN TYPE INTERMEDIATE FILAMENTS IN CULTURES DERIVED FROM HUMAN GLIAL MATERIAL.

Osborn M, Ludwig-Festl M, Weber K, Bignami A, Dahl D, Bayreuther K
Max Planck Inst. Biophysical Chemistry, D-3400 Goettingen, W. Germany
Differentiation; 19(3):161-167 1981

Several cultures established from biopsies of apparently normal adult human glial material showed no cells positive for glial fibrillary acidic protein (GFA) when examined after seven or more cumulative population doublings (CPD), although the established glioma line U251 MG showed approx 3% GFA-positive cells, and U333 CG/343 MG clone 3 showed greater than 98% GFA-positive cells. Both the human glia derived cultures and the glioma lines were positive when assayed with sera specific for vimentin. We therefore investigated the expression of GFA as a function of cumulative population doublings after the establishment of primary cultures. Under our experimental conditions, although GFA-positive cells were clearly present in the primary cultures accounting for some 3%-14% of the cells present, the GFA marker was subsequently lost, and the proliferating cultures expressed only the vimentin type of intermediate filament. Those cells that were GFA-positive also appeared to be vimentin-positive. GFA expression was not reinduced in cultures that had lost the GFA marker by treatment with dibutyryl cyclic AMP. We discuss two alternate hypotheses for the origin of the GFA-negative cells: (1) the cultures are of astrocyte origin but lose the ability to express GFA on culturing; (2) the cultures originate from cells of nonastrocyte origin present in the primary material. (Author abstract) (36 Refs)

8. CONTROL OF PASSIVE PERMEABILITY OF CHINESE HAMSTER OVARY CELLS BY EXTERNAL AND INTRACELLULAR ATP.

Kitagawa T, Akamatsu Y
Dept. Chemistry, Natl. Inst. Health, Shinagawa-ku, Tokyo 141, Japan
Biochim Biophys Acta; 649(1):76-82 1981

External ATP causes passive permeability change in several transformed cells, but not in untransformed cells. We studied the effect of external ATP on the passive permeability of CHO-K1 cells, a transformed clone of Chinese hamster ovary cells. Treatment of the cells with external ATP alone did not produce a permeability change, and this was observed only when a mitochondrial inhibitor, such as rotenone or oligomycin, was present together with ATP. These inhibitors reduced the concentration of intracellular ATP and a permeability change by external ATP was observed when intracellular ATP was decreased more than 70%. This requirement for permeability change of CHO-K1 cells was quite unique, since passive permeability change of other transformed cells so far tested was induced by ATP alone. Treatment of CHO-K1 cells with cyclic AMP analogues increased their sensitivity to external ATP about 2-fold. The roles of external and intracellular ATP in controlling passive permeability are discussed. (Author abstract) (23 Refs)

CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES

9. VASOACTIVE INTESTINAL POLYPEPTIDE MODULATION OF LYMPHOCYTE ADENYLATE CYCLASE.

O'Dorisio MS, Hermina NS, O'Dorisio TM, Balcerzak SP
Dept. Medicine, Ohio State Univ., 410 W. 10th Ave., Columbus, OH, 43210
J Immunol; 127(6):2551-2554 1981

Vasoactive intestinal polypeptide (VIP) was found to be a potent stimulator of lymphocyte adenylate cyclase activity. VIP-induced activation of adenylate cyclase was specific for lymphocytes among peripheral blood cells; ie, VIP did not stimulate the adenylate cyclase activity of neutrophils, monocytes, or platelets. The VIP-induced activation of lymphocyte adenylate cyclase was time, temperature, and concentration dependent. VIP and the GTP analog, Gpp(NH)p, acted synergistically to stimulate lymphocyte adenylate cyclase; stimulation by VIP and PGE1 was additive; and VIP activation was antagonized by somatostatin. VIP-mediated activation of lymphocyte adenylate cyclase was observed in normal human T cells, B cells obtained from a patient with chronic lymphocytic leukemia, and a human T cell culture line. The Raji human B cell culture line did possess adenylate cyclase activity, but this activity was not stimulated by VIP. These results suggest that lymphocytes possess functional receptors for VIP and that this peptide may play a role in modulation of lymphocyte function. (Author abstract) (41 Refs)

10. REMOVAL OF THE SURFACE-BOUND HUMAN CHORIOGONADOTROPIN RESULTS IN THE CESSATION OF HORMONAL RESPONSES IN CULTURED LEYDIG TUMOR CELLS.

Segaloff DL, Ascoli M
Div. Endocrinology, Vanderbilt Univ. Sch. Medicine, Nashville, TN, 37232
J Biol Chem; 256(22):11420-11423 1981

The relationship between the binding and internalization of human choriogonadotropin and the stimulation of cAMP and steroid production was studied in cultured Leydig tumor cells. It was found that removal of the surface-bound hormone results in a rapid cessation of both cAMP and steroid production. We propose that the surface-bound hormone is responsible for the activation of steroidogenesis and that hormone internalization is involved in the deactivation of this process. (Author abstract) (17 Refs)

11. DERMORPHINS, OPIOID PEPTIDES FROM AMPHIBIAN SKIN, ACT ON OPIOID RECEPTORS OF MOUSE NEUROBLASTOMA X RAT GLIOMA HYBRID CELLS.

Glaser T, Hubner K, De Castiglione R, Hamprecht B
(c/o Hamprecht), Physiologisch-Chemisches Institut der Universitat, Koellikerstr, 2, 8700 Wurzburg, W. Germany
J Neurochem; 37(6):1613-1617 1981

Dermorphin and its Hyp6 analogue are opiate-like heptapeptides originally discovered in frog skin and characterized by the presence of a D-Ala2 residue in their sequence. They were assayed for their capacity to compete with [3H]Leu-enkephalin for binding to opioid receptors in membranes of neuroblastoma x glioma hybrid cells. In the presence of 7 nM-[3H]Leu-enkephalin, the concentrations at which they caused 50% inhibition of [3h]enkephalin binding (IC50 values) are 0.1 uM and 0.3 uM, respectively. In contrast, the synthetic L-Ala(2)-dermorphin shows very low affinity for the opioid receptors. In addition, like other opioid peptides, dermorphin and Hyp(6)-dermorphin inhibit the elevation by prostaglandin E1 (PGE1) of the level of cyclic AMP (IC50 values 0.2 uM and 0.4 uM, respectively). The inhibition is prevented by the opiate antagonist naloxone. L-Ala(2)-dermorphin is at least three orders of magnitude less potent in inhibiting the PGE1-evoked increase in the level of cyclic AMP. The results show that peptides with an amino acid sequence quite different from that of the enkephalins can bind to opioid receptors of the hybrid cells. (Author abstract) (29 Refs)

12. RUTHENIUM RED-INSENSITIVE CALCIUM TRANSPORT IN ASCITES-SARCOMA 180/TG CELLS.

Bygrave FL, Anderson TA
Dept. Biochemistry, Faculty Science, Australian Natl. Univ., Canberra, A.C.T. 2600, Australia
Biochem J; 200(2):343-348 1981

Ruthenium Red-insensitive Ca++ transport in the mouse ascites sarcoma 180/TG is enriched in a 'heavy' microsomal fraction (microsomes) sedimented at 35,000 g for 20 min. The subcellular distribution of this Ca++ transport differed from that of Ruthenium Red-sensitive Ca++ transport and (Na+ + K+)-dependent ATPase activity, but was similar to that of glucose 6-phosphatase. The affinity of this transport system for 'free' Ca++ is high (Km approx 6 uM) and that for MgATP somewhat lower (Km approx 100 uM). Ca++ transport by the tumor microsomes, by contrast with that by liver microsomes, was greatly stimulated by low concentrations of Pi. Although incubation of intact ascites cells with glucagon led to an increase in intracellular cyclic AMP, no stable increase in the initial rate of Ca++ transport in the subsequently isolated 'heavy' microsomes could be detected as in similar experiments carried out previously with rat liver cells. Reconstitution experiments suggest that a deficiency exists in the tumor microsomal membrane such as an action of glucagon that is normally present in rat liver microsomes is not evoked. (Author abstract) (20 Refs)

13. THE ACTION OF ADENOSINE ANALOGS ON PC12 CELLS.

Guroff G, Dickens G, End D, Londos C
NIH, NICHD, LDN, Building 6, Room 1A08, Bethesda, MD, 20205
J Neurochem; 37(6):1431-1439 1981

PC12 cells, a nerve growth factor-responsive clone of rat pheochromocytoma, contain a membrane-bound adenylate cyclase, which can be activated by adenosine analogs. The characteristics of the cyclase response indicate the presence of stimulatory adenosine receptors. Adenosine analogs also produce a marked increase in the ornithine decarboxylase levels of the cells, and the characteristics of this response suggest that it is linked to the adenylate cyclase-stimulatory adenosine receptors. The ornithine decarboxylase response elicited by 5'-N-ethylcarboxamideadenosine (NECA), a potent stimulatory adenosine analog, is synergistic with that produced by nerve growth factor. Differentiation of the cells with nerve growth factor, however, does not substantially alter either the response of cyclase to the adenosine analog or the magnitude of the adenosine-evoked ornithine decarboxylase response. Treatment of the cells with NECA produces an increase in the phosphorylation of a specific non-histone nuclear protein. While causing little or no morphological alteration by itself, NECA is synergistic with nerve growth factor in producing neurite outgrowth in PC12 cells. NECA does not cause an induction of acetylcholinesterase in the cells, nor does it appear to affect the induction of this enzyme by nerve growth factor. (Author abstract) (34 Refs)

14. GROWTH OF C6 GLIOMA CELLS IN SERUM-CONTAINING MEDIUM DECREASED BETA-ADRENERGIC RECEPTOR NUMBER.

Dibner MD, Insel PA
Central Res. and Development Dept., Du Pont Experimental Station, Wilmington, DE, 19898
J Cell Physiol; 109(2):309-315 1981

The change of receptors in rat C6 glioma cells grown in serum and serum-free media was characterized and the effects of growth in serum on beta-adrenergic receptor (bAR)-mediated cyclic AMP accumulation was studied. Cells grown in serum-free media had an increased number of bAR. When cells were grown under both conditions for varying lengths of time and measured iodohydroxybenzylpindolol (IHYP)-binding, there was no significant difference in receptor number during the first 3 days in culture; on day 4, and continuing through day 9, the receptor number in the serum-free cells was 30-50% higher than with serum. If the medium was not changed, there was no difference in receptor number between cells grown in serum-containing and serum-free medium for 3 days. When the medium was changed daily, there was a significant increase in the receptor number in cells grown in serum-free medium. Cells grown in serum-free medium had 142% of the bAR measured on cells grown in serum due to an alteration in the number of binding sites per cell. No single factor in the serum-free medium was found to be responsible for an increase in receptor number. Compared with the number of IHYP binding sites on cells grown in serum-free medium, the number of sites was decreased in cells grown with 1% serum. Growth in the presence of serum caused a dose-dependent decrease in the number of bAR, with 1% serum having half the effect of 5 or 10% serum. It was shown that some property of serum leads to a time- and concentration-dependent decrease in bAR number in C6 cells. Growth of C6 cells in serum-containing medium decreased bAR and beta-adrenergic responsiveness by approx the same degree. Boiling failed to inactivate the factor(s) decreasing receptor number in serum, while dialysis partially removed this factor. The presence of serum in medium in which cells are grown can alter properties in the plasma membrane and may alter hormonal responses in such cells. (22 Refs)

CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS

15. CHARACTERISTICS OF THE CYCLIC AMP-PHOSPHODIESTERASE ACTIVATOR IN HUMAN BRAIN TUMORS.

Frattola L, Canal N, Gaini SM, Tonini C, Trabucchi M
Neurological Clinic, Univ. Milan, Bassini Hosp., Via Gorki, 50, 20092 Cinisello Balsamo, Italy
J Neurol Sci; 52(2/3):269-277 1981

The activities of cyclic AMP-phosphodiesterase (PDE) and its endogenous protein activator (PDEA) in normal and tumorous human brain tissue were studied. Samples of normal and pathological brain tissues were obtained from patients with cerebral neoplasia or epilepsy. PDE was purified from the samples. PDE stimulation in the presence of 10 uM Ca++ increased in a linear fashion with increasing amounts of PDEA (up to 8 ug of protein) prepared from normal or tumor tissue. PDEA increased the velocity of the PDE reaction without altering PDE affinity for cyclic AMP (cAMP). PDEA content of normal brain tissue was higher [183 units/mg protein (u/mgp)] than in tumor tissue. PDEA content of tumor tissue by type was astrocytoma 85 u/mgp, glioblastoma 64 u/mgp, medulloblastoma 80 u/mgp, neurinoma, 29 u/mgp, and meningioma 26 u/mgp. Hydrolysis of cAMP occurred at a significantly lower rate in tumor tissue than in normal tissue. PDEA from normal tissue stimulated PDE from normal tissue to a greater extent than PDE from tumor tissues, but tumor PDEA stimulated normal and tumor PDE equally. PDEA lacked tissue specificity. PDE activity in tumors decreased more than PDEA activity; the effect was most evident in tumors of glial origin, which are the most malignant. (34 Refs)

16. CYCLIC NUCLEOTIDES AND CALCIUM IN HUMAN LYMPHOCYTES INDUCED TO DIVIDE.

Rochette-Egly C, Kempf J
Unite de Recherches 61 de l'INSERM, avenue Moliere, F-67200 Strasbourg, France
J Physiol (Paris); 77(6/7):721-725 1981

Intracellular concentrations of cyclic AMP (cAMP), and cyclic guanosine monophosphate (cGMP) were measured in lymphocytes triggered by concanavalin A, phytohemagglutinin, 12-O-tetradecanoylphorbol-13-acetate and the calcium ionophore A 23187. cGMP rose within min without concomitant cAMP alteration. The cAMP and cGMP levels rose during the prereplicative and replicative phases, respectively. Under calcium depleting conditions, the fluctuations in cAMP and cGMP and increase in (3H)thymidine incorporation into DNA were abolished, suggesting a role for Ca++ in the regulation of lymphocyte proliferation. (21 Refs)

CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES

17. THE RELATIONSHIP BETWEEN PLASMA MEMBRANE LIPID COMPOSITION AND PHYSICAL-CHEMICAL PROPERTIES. II. EFFECT OF PHOSPHOLIPID FATTY ACID MODULATION ON PLASMA MEMBRANE PHYSICAL PROPERTIES AND ENZYMATIC ACTIVITIES.

Poon R, Richards JM, Clark WR
Jewish Hosp. St. Louis, 216 S. Kings Highway, St. Louis, MO, 63178
Biochim Biophys Acta; 649(1):58-66 1981

The fatty acid composition of plasma membrane phospholipids of the murine T lymphocyte tumor EL4 were systematically modified in an attempt to understand the relationship between lipid bilayer composition and plasma membrane physical and biological properties. Two plasma membrane enzyme activities, adenylate cyclase and ouabain-sensitive (Na+ + K+)-ATPase, were measured in normal and fatty acid-substituted EL4 plasma membrane fractions. The fatty acid effect on enzyme activities was similar to previously reported effects of fatty acids on cytotoxic T cell function. The activity of both enzymes was inhibited by saturated fatty acids, while unsaturated fatty acids had a moderate enhancing effect on both enzyme activities. Using two different nitroxide derivatives of stearic acid, the order parameter and approx rotational correlation times were calculated from ESR spectra of normal and fatty acid-modified plasma membranes. No significant difference was found in either parameter in these membranes. These results, in conjunction with earlier data from our laboratory and others, suggest that caution should be exercised in inferring changes in membrane 'fluidity' based on lipid modulation of biological membranes. (Author abstract) (38 Refs)

18. EARLY BIOCHEMICAL REACTIONS IN THE LUNG OF SHEEP EXPOSED TO ASBESTOS: EVIDENCE FOR CYCLIC AMP ACCUMULATION IN BRONCHOALVEOLAR LAVAGE FLUIDS.

Lemaire I
Unite de Recherche Pulmonaire, Faculte de medicine, Universite de Sherbrooke, Sherbrooke, Quebec J1H 5N4, Canada
Lung; 159(6):323-332 1981

The effects of asbestos exposure on the bronchoalveolar milieu of sheep were studied. Asbestos (0, 1, 2, 8, or 16 mg) was suspended in 50 ml phosphate-buffered saline and administered intratracheally once/mo for 3 mo. After 3 mo the 1 and 8 mg doses were raised to 64 and 128 mg, respectively, for 3 mo. The other doses remained unchanged. No significant changes in pulmonary function were noted. The only significant histological change was the presence of occasional fibers in asbestos-exposed animals (AA). Cells obtained from bronchoalveolar lavage (BAL) showed no change in total cell yield or numbers of macrophages, lymphocytes, neutrophils, or epithelial cells. Asbestos exposure produced eosinophilia in most cases. BAL fluid from AA contained elevated levels of total protein, albumin, alpha-1-globulin, and cyclic AMP.

These changes may reflect chronic inflammatory reaction and fibrogenic response to asbestos. (35 Refs)

19. EFFECTS OF THE PHORBOL ESTER 4-O-METHYL-12-O-TETRADECANOYLPHORBOL-13-ACETATE ON MOUSE SKIN IN VIVO: EVIDENCE FOR ITS USELESSNESS AS A NEGATIVE CONTROL COMPOUND IN STUDIES ON THE BIOLOGICAL EFFECTS OF PHORBOL ESTER TUMOR PROMOTERS.

Furstenberger G, Richter H, Argyris TS, Marks F
Deutsches Krebsforschungszentrum, Institut fur Biochemie, Im Neuenheimer Feld 280 D-6900 Heidelberg, D-6900 Heidelberg, W. Germany
Cancer Res; 42(1):342-348 1982

Compared to 12-O-tetradecanoylphorbol-13-acetate (TPA), the phorbol ester 4-0-methyl-12-0-tetradecanoylphorbol-13-acetate (4-O-methyl-TPA) is only a weak skin irritant and tumor promoter. When topically applied to mouse skin, 400 nmol 4-O-methyl-TPA induce hyperproliferation of epidermis in vivo to approx the same extent as do 10 nmol TPA. However, in contrast to TPA, 4-O-methyl-TPA-induced proliferation is not followed by sustained epidermal hyperplasia. In addition, the methyl ether of TPA does not cause early prostaglandin accumulation, and its hyperproliferative effect is insensitive to indomethacin inhibition. Contrary to the action of TPA, 4-O-methyl-TPA induces neither an early increase in the rate of phospholipid turnover nor an early increase in ornithine decarboxylase activity or polyamine accumulation in epidermis and does not desensitize epidermis to the effects of beta-adrenergic agonists and epidermal G chalone. Finally, 4-O-methyl-TPA is only a weak stimulator of epidermal cyclic AMP phosphodiesterase activity. Consequently, it is concluded that the mechanism of action of 4-O-methyl-TPA as a skin mitogen is different from that of TPA. Whereas TPA evokes prostaglandin-dependent and chalone-insensitive hyperplastic development of epidermis (hyperplastic transformation), ie, probably a relapse to the neonatal state, 4-O-methyl-TPA apparently stimulates nothing but normal everyday tissue regeneration. In this respect, treatment with 4-O-methyl-TPA resembles a non-damaging stimulus of epidermal cell proliferation (observed, for example, with skin massage), whereas TPA evokes a response which is characteristic for epidermal injury. Considering these fundamental differences in biological action between TPA and its methyl ether, 4-O-methyl-TPA is concluded to be an unsuitable negative control compound for studies on the biological effects of phorbol ester tumor promoters. (Author abstract) (41 Refs)

PROTEIN KINASE STUDIES

20. EVIDENCE THAT VIRAL TRANSFORMING GENE PRODUCTS AND EPIDERMAL GROWTH FACTOR STIMULATE PHOSPHORYLATION OF THE SAME CELLULAR PROTEIN WITH SIMILAR SPECIFICITY.

Erikson E, Shealy DJ, Erikson RL
Dept. Pathology, Univ. Colorado Sch. Medicine, Denver, CO, 80262
J Biol Chem; 256(22):11381-11384 1981

Treatment of A-431 human epidermoid carcinoma cells with epidermal growth factor (EGF) was shown to enhance the phosphorylation of a Mr = 34,000 protein. Because the phosphorylation of an analogous protein is enhanced in various cell lines transformed by Rous sarcoma virus (RSV) we characterized the phosphorylation of the A-431 Mr = 34,000 protein under these two conditions in order to determine whether there are common pathways between viral transformation and EGF stimulation. The results of tryptic phosphopeptide mapping and phosphoamino acid analysis showed that the Mr = 34,000 protein was phosphorylated in an identical manner by the EGF-stimulated protein kinase activity and by the protein kinase activity of the RSV transformation-specific protein or of its normal cell homolog. Although the specific protein kinase that phosphorylates the Mr = 34,000 protein under conditions of EGF-stimulation is not yet identified, these studies demonstrate that at least one consequence of EGF stimulation is identical with one of the consequences of viral transformation. (Author abstract) (43 Refs)

21. THE SRC GENE PRODUCT (PP60SRC) OF AVIAN SARCOMA VIRUS RAPIDLY INDUCES DNA SYNTHESIS AND PROLIFERATION OF CALCIUM-DEPRIVED RAT CELLS.

Durkin JP, Boynton AL, Whitfield JF
Animal and Cell Physiology Group, Natl. Res. Council Canada, Ottawa, Ontario K1A OR6, Canada
Biochem Biophys Res Commun; 103(1):233-239 1981

Calcium-deprived tsLA23-NRK cells are reported to be blocked near the G1/S boundary of the cell cycle at 40 C, a temperature non-permissive for neoplastic transformation. Such arrested cells rapidly initiated DNA synthesis and resumed proliferating in calcium-deficient medium when the temperature was lowered to 36 C, a level permissive for the production of active pp60src and neoplastic transformation. In contrast, previous studies had shown that calcium-deprived wtB77-NRK cells grew rapidly and continuously at either 40 C or 36 C, while uninfected NRK cells were unable to proliferate at either temperature in calcium-deficient medium. The product of the src transforming gene of avian sarcoma virus may thus act near the G1/S border to rapidly bypass or directly stimulate a normally calcium-dependent process needed for the initiation of DNA synthesis. pp60src thus allows neoplastic, avian sarcoma virus-infected NRK cells to proliferate in calcium-deficient medium which does not support the proliferation of non-neoplastic counterparts. This work supports the suggestion that changes in protein kinase activities are responsible for the ability of a wide variety of transformed avian, bovine, rodent and human cells to proliferate in calcium-deficient medium. (21 Refs)

22. TYROSYLPROTEIN KINASE AND PHOSPHATASE ACTIVITIES IN MEMBRANE VESICLES FROM NORMAL AND ROUS SARCOMA VIRUS-TRANSFORMED RAT CELLS.

Gallis B, Bornstein P, Brautigan DL
Dept. Biochemistry, Univ. Washington, Seattle, WA, 98195
Proc Natl Acad Sci USA; 78(11,part2):6689-6693 1981

Membrane vesicles, isolated from normal and Rous sarcoma virus-transformed rat cells, have an associated cyclic-AMP independent kinase that phosphorylates a Mr 37,000 protein in vesicles from normal cells and proteins of Mr 37,000, 50,000, and 67,000 in vesicles from transformed cells. Proteins in vesicles from normal and transformed cells contain 9% and 77%, respectively, of their labeled phospho amino acids as phosphotyrosine. Thus, isolation of vesicles and subsequent incubation with [gamma-32P]ATP enriches the proportion of labeled phosphotyrosine in proteins (relative to other phospho amino acids) by two orders of magnitude over that found in intact cells. The in vitro phosphorylation of each of these proteins is enhanced in the presence of 10 uM Zn^{++}, a phosphotyroslyprotein phosphatase inhibitor. From these studies it appears that membrane vesicles may be a valuable system for examination of transformation-specific phosphorylation of proteins. (Author abstract) (25 Refs)

23. NUCLEAR PROTEIN KINASES OF GH3 CELLS.

Fabbro D, Jungmann RA, Eppenberger U
Hormone Lab. and Experimental Endocrinology, Univ. Clinic Medical Sch., CH-4031 Basel, Switzerland
Horm Res; 14(4):260-276 1981

Three distinct cAMP-independent nuclear protein kinase activities (PCv, PC-II and PC-III) have been separated by phosphocellulose chromatography of GH3 nuclear NaCl extracts. The kinase activities exhibit distinct substrate specificities and preference for either the basic substrates protamine sulfate and histones H1 and H3 (kinase activity PCv) or the acidic protein substrates casein (PC-II) and phosvitin (PC-III). The three nuclear protein kinases further differ in their estimated mol wt, apparent Km values and in their sensitivity toward NaCl. All three kinases require Mg^{++} for optimal activity. (Author abstract) (40 Refs)

24. PROTEIN KINASES ASSOCIATED WITH THE ADENOVIRUS SINGLE-STRANDED DNA-BINDING PROTEIN.

Cajean-Feroldi C, Loeb J, Meguenni S, Girard M
Unites de Physiologie des Virus et de Biochimie des Regulations, Institut de Recherches Scientifiques sur le Cancer, Boite postale 8, F-94800 Villejuif, France
Eur J Biochem; 120(1):79-87 1981

Protein kinase activities copurifying with the 72,000-Mr DNA-binding protein of adenovirus on DNA-cellulose chromatography and gel filtration in acrylamide/agarose have been partially characterized and purified. One of these kinases was found to phosphorylate efficiently the viral DNA-binding protein in vitro and to be stimulated severalfold by the addition of histones, protamine, or polyamines. The kinase does not, however, phosphorylate histones, protamine, casein, or phosvitin. A second protein kinase was also recovered from single-stranded DNA-cellulose which is able to phosphorylate the 72,000-Mr DNA-binding protein, but which is inhibited by the addition of histones. Phosphorylation in vitro of the 72,000-Mr DNA-binding protein from the ts125 mutants of adenovirus by the histone-stimulated protein kinase was found to be thermosensitive. (Author abstract) (52 Refs)

25. ASSOCIATION OF MICROTUBULES AND INTERMEDIATE FILAMENTS IN NORMAL FIBROBLASTS AND ITS DISRUPTION UPON TRANSFORMATION BY A TEMPERATURE-SENSITIVE MUTANT OF ROUS SARCOMA VIRUS.

Ball EH, Singer SJ

Dept. Biochemistry, Univ. Western Ontario, London, Ontario, Canada N6A 5C1

Proc Natl Acad Sci USA; 78(11,part2):6986-6990 1981

By double indirect immunofluorescence, using primary rabbit antibodies to tubulin and guinea pig antibodies to vimentin, we have simultaneously labeled microtubules and intermediate filaments in several types of cultured normal fibroblasts. With well-spread interphase cells there was an extensive but not complete correspondence of the labeling patterns for the two filamentous structures out to the cell periphery. This correspondence existed both at a gross level, where parallel but not coincident arrays of thickly labeled strands of the two types of filaments were observed, and at a fine level, where thinly labeled strands of the two were superimposed. The results suggest that there may be some type(s) of molecular linkages between microtubules and vimentin intermediate filaments that is under metabolic control. With NRK fibroblasts infected with a temperature-sensitive mutant (LA23) of Rous sarcoma virus, cells grown at the nonpermissive temperature (39 C) showed the correspondence of the distributions of the microtubules and intermediate filaments characteristic of the normal phenotype but within 1 hr after a shift to the permissive temperature (33 C) there was an extensive retraction of the intermediate filaments around the cell nucleus whereas the microtubules remained dispersed into the cell periphery. These results suggest that one of the functions carried out by p60src, the protein kinase responsible for transformation by Rous sarcoma virus, may be to modify the component(s) involved in the putative linkages between microtubules and intermediate filaments in the normal cells. (Author abstract) (26 Refs)

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AUTHOR INDEX WITH CITATION NUMBERS

Akamatsu Y 8
Anderson TA 12
Argyris TS 19
Ascoli M 10
Azarnia R 6
Balcerzak SP 9
Ball EH 25
Bayreuther K 7
Bershtein LM 4
Bignami A 7
Bornstein P 22
Boynton AL 21
Brautigan DL 22
Bygrave FL 12
Cajean-Feroldi 24
Canal N 15
Chawla MS 1
Chuman L 3
Clark WR 17
Creech RH 2
Currie ET 1
Dahl D 7
Dahl G 6
De Castiglione 11
Dibner MD 14
Dickens G 13
Diekamp U 1
Durkin JP 21
End D 13
Eppenberger U 23
Erikson E 20
Erikson RL 20
Fabbro D 23
Frattola L 15
Furstenberger G 19
Gaini SM 15
Galkina LA 5
Gallis B 22
Girard M 24
Glaser T 11
Guroff G 13
Hamprecht B 11
Hermina NS 9
Hubner K 11
Insel PA 14
Jungmann RA 23
Kempf J 16
Kitagawa T 8
Kucherenko NE 5
Kukla LJ 1
Lad TE 1
Larson CS 1
Lemaire I 18
Lemekhov VG 4
Loeb J 24
Loewenstein WR 6
Londos C 13
Ludwig-Festl M 7
Marks F 19
McGuire WP 1
Meguenni S 24
Mehta CR 2
Nelson RB 1
O'Dorisio MS 9
O'Dorisio TM 9
Osborn M 7
Poon R 17
Richards JM 17
Richter H 19
Rindler MJ 3
Rochette-Egly C 16
Ruckdeschel JC 2
Saier MH 3
Salazar OM 2
Sarma PR 1
Sato G 3
Segaloff DL 10
Seleznev IK 4
Shealy DJ 20
Singer SJ 25
Sponzo RW 2
Taub M 3
Tichler T 1
Tonini C 15
Trabucchi M 15
Tsudzevich BA 5
U B 3
Weber K 7
Whitfield JF 21
Zawila P 1

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In this issue:

- **REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES** **1-2**
- **REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES** **3-4**
- **CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES** **5-9**
- **CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS** **10-12**
- **CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES** **13-16**
- **PROTEIN KINASE STUDIES** **17-22**

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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HE 20. 3173/2:

REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES

1. IMMUNOLOGICAL, BIOCHEMICAL, ULTRASTRUCTURAL, AND ELECTROPHYSIOLOGICAL CHARACTERISTICS OF A HUMAN GLIOBLASTOMA-DERIVED CELL CULTURE LINE.

Black PM, Kornblith PL, Davison PF, Liszczak TM, Merk LP, Smith BH, McKeever PE, Quindlen EA
Neurosurgical Service, Massachusetts General Hosp., Boston, MA, 02114
J Neurosurg; 56(1):62-72 1982

This report presents the results of a study using multiple techniques of the established human cell line, LM, which has been developed in culture medium from a patient with a right temporoparietal glioblastoma. This cell line has human subtetraploid karyotype and has several features of a transformed line in culture. These include continuous propagation for 10 years, ability to form tumor nodules when transplanted into immunologically suppressed hamsters, and pleomorphic appearance. Ultrastructurally, it is characterized by multiple nuclei, few actin cables, and numerous surface-membrane microvilli, as well as abundant 9-to 10-nm cytoplasmic filaments. By its immunological reactivity, the line can be shown to contain glial fibrillary acidic protein at low levels, consistent with its glial origin and continued nature. Dibutyryl cyclic adenosine monophosphate (db-cAMP) induces formation of long astrocytic-like processes as well. Its membrane electrical characteristics include a low resting membrane potential and short time constant. Used in a microtiter antiglioma antibody cytotoxicity assay, LM yields a positive reaction to antibodies in the sera of 80% of patients with astrocytomas and only 9% of normal blood-bank donors, suggesting that it shares common antigens with other astrocytic tumor lines. The varied characteristics of this glioblastoma-derived line emphasize the 'multiforme' nature of the neoplasm and suggest that for characterization of any such line, multiple parameters are necessary to allow comparison with other long-term glioblastoma lines in the literature. The usefulness of the LM line in in vitro cell biological, immunological, chemotherapeutic, and radiobiological studies of gliomas makes such efforts very worthwhile. (Author abstract) (58 Refs)

2. ALTERATIONS IN CALCITONIN AND PARATHYROID HORMONE RESPONSIVENESS OF ADENYLATE CYCLASE IN F9 EMBRYONAL CARCINOMA CELL TREATED WITH RETINOIC ACID AND DIBUTYRYL CYCLIC AMP.

Evain D, Binet E, Anderson WB
Unite' INSERM 188, 74 Av. Denfert Rochereau, 75014 Paris, France
J Cell Physiol; 109(3):453-459 1981

Adenylate cyclase (AC) was assayed by measuring conversion of (alpha-32P) ATP to cyclic (32P) AMP at 37 C in F9 embryonal carcinoma cells induced to differentiate to endodermal cells by exposure to 30 nanog/ml retinoic acid. Basal, guanosine triphosphate-, and fluoride-stimulated AC activities increased as induced change in cells occurred. The endodermal cell type showed little AC stimulation in response to calcitonin, and a strong increase in parathyroid hormone response. This behavior is the opposite of that in untreated F9 cells, which show greatest cyclic AMP stimulation with calcitonin, and little with parathyroid hormone. Treatment of retinoic acid-generated endodermal cells with dibutyryl cyclic AMP yields a type with neural-like morphology. AC in these cells was stimulated by parathyroid hormone, prostaglandin E1, isoproterenol, and epinephrine, but calcitonin response was lost. The possible metabolic role during development for calcitonin and parathyroid hormone indicated by these results implies that changes in AC hormonal responsiveness could serve as markers of early differentiation stages. (25 Refs)

REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES

3. CYCLIC AMP REGULATION OF LACTATE DEHYDROGENASE. QUANTITATION OF LACTATE DEHYDROGENASE M-SUBUNIT MESSENGER RNA IN ISOPROTERENOL- AND N6,O(2)'-DIBUTYRYL CYCLIC AMP-STIMULATED RAT C6 GLIOMA CELLS BY HYBRIDIZATION ANALYSIS USING A CLONED CDNA PROBE.

Miles MF, Hung P, Jungmann RA
(c/o Jungmann) Cancer Center, Dept. Molecular Biology, Northwestern Univ. Medical Sch., Chicago, IL, 60611
J Biol Chem; 256(23):12545-12552 1981

We have cloned DNA complementary to mRNA coding for rat C6 glioma cell lactate dehydrogenase M-subunit. Double-stranded DNA complementary to a portion of lactate dehydrogenase mRNA was inserted into the Pst I site of plasmid pBR322 by the dC.dG tailing technique and amplified in Escherichia coli HB101. A recombinant plasmid containing lactate dehydrogenase cDNA was identified by colony hybridization to a cDNA prepared from partially purified lactate dehydrogenase mRNA and by hybridization-selected translation. The recombinant plasmid (pRLD42) contains a 680 nucleotide insert of lactate dehydrogenase mRNA. Hybridization of nick-translated pRLD42 to glioma cell poly(A)+RNA separated on agarose gel and transferred to nitrocellulose exhibited Mr = 5.9 x 10(5) for lactate dehydrogenase mRNA. Furthermore, Northern blot analysis of RNA from unstimulated and isoproterenol-stimulated glioma cells indicated a two-fold increase of lactate dehydrogenase mRNA molecules in stimulated cells. The two-fold increase of lactate dehydrogenase mRNA was confirmed by RNA-excess kinetic hybridization using pRLD42 DNA and poly(A)+RNA from unstimulated isoproterenol-, and dibutyryl cAMP-stimulated glioma cells. These data demonstrate that isoproterenol and dibutyryl cAMP cause an increase of the number of lactate dehydrogenase M-subunit mRNA molecules in glioma cells which, in part, determines the extent of synthesis of the lactate dehydrogenase M-subunit. (Author abstract) (56 Refs)

4. ALKALINE PHOSPHATASE INHIBITION BY PARATHYROID HORMONE AND ISOPROTERENOL IN A CLONAL RAT OSTEOSARCOMA CELL LINE. POSSIBLE MEDIATION BY CYCLIC AMP.

Majeska RJ, Rodan GA
Dept. Oral Biology, Univ. Connecticut, Sch. Dental Medicine, Farmington, CT, 06032
Calcif Tissue Int; 34(1):59-66 1982

The effect of parathyroid hormone (PTH 1-34 bovine) on alkaline phosphatase activity was investigated in an osteoblast-like clonal cell line derived from rat osteosarcoma (ROS 17/2). The properties of ROS 17/2 alkaline phosphatase resembled those of alkaline phosphatase from bone and from both human and mouse osteosarcoma cells. Levamisole inhibition and electrophoretic mobility resembled the rat bone enzyme activities. Incubation for 15 min at 56 C destroyed 98% of the alkaline phosphatase activity in butanol-extracted rat bone homogenate, and 85% of that in a similarly treated ROS 17/2 particulate fraction. The specific activity of ROS 17/2 alkaline phosphatase increased with time in culture. The increase was inhibited by PTH 1-34 and (-)-isoproterenol in a dose-dependent manner, starting at near-physiological hormone concentration (measured 48 hr after hormone addition). The ID50 values were 0.02 nano (n)M for PTH 1-34 and 1.7 nM for isoproterenol. The two hormones stimulated ROS 17/2 adenylate cyclase at higher concentrations: 13 nM for PTH 1-34 and 16 nM for isoproterenol. The rise in alkaline phosphatase was also inhibited by dibutyryl cyclic AMP and 8-bromo-cyclic AMP (0.1 milliM). Results suggest that PTH inhibition of alkaline phosphatase represents a physiological response to the hormone in the ROS 17/2 cell line; the findings implicate cyclic AMP as a mediator of the PTH effect. (43 Refs)

CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES

5. CALCITONIN INDUCTION OF A PERSISTENT ACTIVATED STATE OF ADENYLATE CYCLASE IN HUMAN BREAST CANCER CELLS (T 47D).

Lamp SJ, Findlay DM, Moseley JM, Martin TJ
(c/o Martin) Dept. Medicine, Univ. Melbourne, Repatriation General Hosp., Heidelberg, Victoria 3081, Australia
J Biol Chem; 256(23):12269-12274 1981

In a calcitonin-responsive human breast cancer cell line (T 47D), specific receptors exist for calcitonin in intact cells, which show saturable binding which is only slowly and incompletely reversible. Full stimulation of cyclic AMP production in the cells was achieved with less than 50% receptor occupancy. Pretreatment of cells in culture with calcitonin, followed by washing, led to loss of adenylate cyclase response to calcitonin in homogenates. This took place rapidly, reaching a maximum after 15 min of preincubation with 3 nM salmon calcitonin, and was associated with a substantial increase in basal adenylate cyclase activity, which was dose-dependent and agonist-specific, in that it did not occur upon treatment of the cells with prostaglandin E2. Receptor loss, as measured by decrement in specific binding of [125I]calcitonin to intact cells, took place more slowly, requiring at least 1 hr for completion. Recovery of adenylate cyclase responsiveness and return of basal activity to normal was a relatively slow process, always requiring more than 24 hr for completion. Neither the desensitization nor the persistent basal activity could be transferred in the cytosol compartment of calcitonin-preincubated cells. When calcitonin-preincubated cells were washed, and at intervals thereafter, the production of cyclic AMP was measured in the presence of a phosphodiesterase inhibitor, a 120-fold rise in basal cyclic AMP production was observed, and the response to added calcitonin was lost. Return of basal activity and of calcitonin responsiveness followed a time course similar to that seen in recovery of adenylate cyclase response. The results indicate the ability of calcitonin to induce rapidly a persistent state of adenylate cyclase activation which remains stable for several hours. This may be due to persistent tight binding of calcitonin but may also be related to changes induced by calcitonin in availability or function of nucleotide regulatory units. (Author abstract) (34 Refs)

6. EFFECT OF 12-O-TETRADECANOYL-PHORBOL-13-ACETATE ON CA2+ EFFLUX AND PROTEIN DISCHARGE IN PANCREATIC ACINI.

Gunther GR
Dept. Biochemistry, St. Jude Children's Res. Hosp., P. O. Box 318, Memphis, TN, 38101
J Biol Chem; 256(23):12040-12045 1981

When pancreatic acini are exposed to the peptide cerulein (an analogue of the hormone cholecystokinin) or the cholinergic agonist carbamylcholine, they exhibit a rapid release of intracellular Ca++ and a rise in the level of cGMP, accompanied by discharge of secretory proteins. The phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA) stimulates protein discharge without affecting the level of cGMP, while N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) elevates cGMP, but does not elicit discharge. To clarify the relationships among these events, the effect of TPA and MNNG on Ca++ efflux in guinea pig acini was determined. Cells were incubated in medium containing 45Ca++ and transferred to medium with nonradioactive Ca++ before adding the agents to be tested. Cerulein (10(-9) M), carbamylcholine (10(-5) M), and the Ca++ ionophore A23187 (5 ug/ml) all caused the release of 40-60% of the cell-associated 45Ca++ within 3 min. In contrast, acini incubated with 10(-7) M TPA, 10(-4) M MNNG, or no added agents retained over 80% of their 45Ca++ after 22 min. TPA caused significant secretory discharge within the first 15 min and did not block Ca++ efflux stimulated by other secretagogues. TPA-induced discharge requires energy and the presence of sufficient Ca++ in the extracellular medium. These observations are consistent with a model in which the rapid release of Ca++ into the cytoplasm triggers an increase in the level of cGMP and, by a separate series of events, protein discharge. TPA may act at a later point on the pathway to discharge, but before an obligatory step requiring Ca++. (Author abstract) (31 Refs)

7. COMPARISON OF PATHOLOGICAL AND BIOCHEMICAL STUDIES OF TRANSPLANTED MORRIS HEPATOMAS.

Slaughter L, Morris HP, Criss WE
Howard Univ., Coll. Medicine, Washington, DC, 20059
Adv Enzyme Regul; 19:19-25 1981

Ten consecutive generations of each of 57 hepatoma cell lines, 5,000 transplantable tumors, have been monitored to define their salient morphologic features. Also the lungs of rats bearing these tumors were evaluated for evidence of transplantable tumor cellular metastases. These evaluations have led us to conclude the following. (a) The pattern of tumor cell growth in uniform and consistent from generation to generation. (b) Tumor cell pleomorphism is extremely variable. (c) All tumors are well vascularized. (d) Mitoses are variable between generations. (e) Varying amounts of necrosis are always present in all tumor cells lines. (f) The degree of differentiation is uniform in a given tumor line. (g) All well differentiated transplantable tumors metastasize to normal lungs but not to inflamed lungs. (h) Undifferentiated or poorly differentiated transplantable tumors cells rarely metastasize to the lungs. Four major program areas are reviewed. (a) Several of the Morris hepatomas may be hormone dependent or hormone responsive with regard to glucagon, insulin, cortisol or thyroxine. (b) The feedback regulatory control in cholesterol synthesis, which is missing in every Morris hepatoma, is probably located at the hydroxymethylglutaryl-CoA reductase site of action. (c) The intracellular 'second messenger' system in the hepatomas shows lost responsiveness of the cyclic AMP subsystem and increased responsiveness of the cyclic AMP subsystem and increased responsiveness of the cyclic GMP subsystem. (d) The major factors in determining a tumor tissue's response to radiation and/or chemotherapy are cell doubling times, cell loss factors, cellular growth fractions, and cell cycle intervals. (Author abstract) (28 Refs)

8. DIFFERENCE BETWEEN MAGNESIUM AND MANGANESE IONS IN MODIFICATION OF EFFECT OF CATECHOLAMINE ON ADENYLATE CYCLASE SYSTEM IN EHRLICH ASCITES TUMOR CELLS.

Kurokawa T, Kitamura Y, Moriuchi M, Ishibashi S

Dept Physiological Chemistry, Inst. Pharmaceutical Sciences, Hiroshima Univ. Sch. Medicine, Minami-ku, Hiroshima 734, Japan

J Pharmacobio Dyn; 4(10):794-797 1981

Basal and norepinephrine-stimulated adenylate-cyclase activities in Ehrlich ascites tumor cells were measured in the presence of various concentrations of either Mg+ + or Mn+ +. Both activities increased gradually with increase in Mg+ + concentration. Both activities were max in the presence of low Mn+ + concentrations, and decreased with increase in concentration. The decrease in activity was more pronounced in the norepinephrine-stimulated activity. Similar difference between the two divalent cations was observed in the effect on binding of the beta-adrenergic ligand to the membrane receptor. (13 Refs)

9. HETEROGENEOUS FORMS OF THYROID-STIMULATING HORMONE IN MOUSE THYROTROPIC TUMOR AND SERUM: DIFFERENCES IN RECEPTOR-BINDING AND ADENYLATE CYCLASE-STIMULATING ACTIVITY.

Pekonen F, Carayon P, Amr S, Weintraub BD

(c/o Weintraub), NIH, Building 10, Room 8N315, Bethesda, MD, 20205

Horm Metab Res; 13(11):617-620 1981

Thyroid-stimulating hormone (TSH) in tumor and serum of mice with thyrotropic tumors was studied by radioimmunoassay (RIA), radioreceptor assay (RRA) and thyroid adenylate cyclase assay (ACA). In unfractionated samples, serum TSH displayed significantly higher ACA/RIA (0.69 +-0.10) and ACA/RRA ratios (0.79 +-0.04) than TSH in tumor extracts (0.45 +-0.05 and 0.31 +-0.01, respectively). After gel chromatography, both tumor and serum TSH activity measured in RIA and RRA eluted in broad, heterogenous peaks with an apparent mol wt range of 26,000-44,000 daltons. The ACA activity of tumor TSH eluted in two sharp peaks (26,000 and 44,000) while that of serum TSH eluted in three peaks (26,000, 33,000 and 44,000). The ACA/RIA and ACA/RRA ratios varied greatly among the chromatography fractions, the lowest ratios being detected in the tumor TSH of 33,000 daltons (0.02 for each) and the highest ratios in both tumor and serum TSH of 26,000 (ACA/RIA = 1.60-1.90, ACA/RRA = 1.12-1.20). Since previous biosynthetic studies have suggested that such heterogeneous forms of mouse tumor TSH differ solely in carbohydrate content, our data suggest that the biologic activity of TSH may be modulated by its glycosylation. (Author abstract) (21 Refs)

CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS

10. RAPID INCREASE IN GUANOSINE 3',5'-CYCLIC-MONOPHOSPHATE IN INTERFERON-TREATED MOUSE LEUKEMIA L1210 CELLS: RELATIONSHIP TO THE DEVELOPMENT OF THE ANTIVIRAL STATE AND INHIBITION OF CELL MULTIPLICATION.

Tovey MG, Rochette-Egly C

Institut de Recherches Scientifigues sur le Cancer, Villejuif, France

Virology; 115(2):272-281 1981

Treatment of mouse leukemia L1210 cells with electrophoretically pure mouse interferon caused a rapid (1 to 5 min) and transitory (duration 5 to 10 min) increase in the intracellular concentration of guanosine 3',5'-cyclic monophosphate (cyclic GMP). No significant increase in the intracellular concentration of adenosine 3',5'-cyclic monophosphate (cyclic AMP) was observed until 2 to 3 hr after the addition of interferon. Electrophoretically pure mouse interferon caused a dose-dependent increase in the intracellular concentration of cyclic GMP at concentrations ranging from 1.0 to 10(4) units/ml. The interferon-induced increase in cyclic GMP was abrogated by specific antibody to mouse interferon. Furthermore, interferon had no effect on the intracellular concentration of cyclic GMP in a line of L1210 cells resistant to the action of interferon. The interferon-induced increase in cyclic GMP was calcium dependent and was inhibited by indomethacin and aspirin at concentrations of 10(-7) to 10(-5) M. Depletion of intracellular calcium prior to the addition to interferon abrogated the interferon-induced increase in cyclic GMP without preventing either the development of the antiviral state or the inhibition of cell multiplication in interferon-treated L1210 cells. These results suggest that cyclic GMP may not mediate these two biologic effects of interferon but may rather reflect rapid changes in interferon-treated cells. (Author abstract) (29 Refs)

11. USE OF CHEMOSTAT CULTURE FOR THE STUDY OF THE EFFECT OF INTERFERON ON TUMOR CELL MULTIPLICATION.

Tovey MG

Lab. Viral Oncology, BP8, Institut de Recherches Scientifiques sur le cancer, 94800 Villejuif, France

Methods Enzymol; 79:391-404 1981

Restrictions of the conventional method for culture of animal cells in batches are outlined, and an alternative system, continuous-flow chemostat culture, is described, which overcomes many of its disadvantages. In practice, a chemostat culture consists of a culture of fixed vol, into which nutrient medium is fed at a constant rate. Techniques for establishment and characterization of mouse leukemia L1210 cells are described in detail. Methods for study of the effects of interferon on multiplication of chemostat-cultured L1210 cells are reviewed. These studies include: cellular macromolecular synthesis and nucleotide transport (measured by 3H-thymidine and 3H-uridine incorporation); intracellular cyclic nucleotide concentrations measured by protein-binding assay; interferon sensitivity at different rates of cell multiplication; and the effect of interferon on cellular metabolism as measured by microcalorimetry. The technique has many uses, and is applicable to study of cells of rabbit, hamster and human origin, as well as cells from other mouse lines. (50 Refs)

12. A NEW IN VITRO ASSAY FOR HUMAN THYROID STIMULATOR USING CULTURED THYROID CELLS: EFFECT OF SODIUM CHLORIDE ON ADENOSINE 3',5'-MONOPHOSPHATE INCREASE.

Kasagi K, Konishi J, Iida Y, Ikekubo K, Mori T, Kuma K, Torizuka K
(c/o Konishi) Dept. Nuclear Medicine, Kyoto Univ. Sch. Medicine, Shogoin, Sakyo-ku, Kyoto 606, Japan
J Clin Endocrinol Metab; 54(1):108-114 1982

A new sensitive in vitro assay for human thyroid stimulator (HTS) was developed using human thyroid adenoma cells in monolayer culture. After being cultured for 2 days, the cells were incubated in 0.3 ml Hank's solution without 0.8% NaCl (medium 1) and with thyroid stimulator (bovine TSH or 3 mg patient serum immunoglobulin G) at 37 C for 2 hr. The cAMP generated in the cells and the medium during the incubation was measured by RIA. The assay was sensitive enough to elicit a 1.7-to 7.9-fold increase in cAMP at a TSH concentration of 10 uU/ml. HTS was detected in 33 (82.5%) of the 40 patients with untreated Graves' disease using this assay system. In Hank's solution (medium 2), however, HTS was detected in only 5 (23.8%) of the 21 patients with untreated Graves' disease. cAMP increment upon stimulation by either TSH or HTS in medium 1 was larger than that in medium 2, and the difference in the response to HTS using the two media was much greater than that in the response to TSH. Therefore, all HTS-immunoglobulin G studied sh owed higher activity using medium 1 than using medium 2 when expressed as bovine TSH equivalent. Analysis by the Lineweaver-Burk plot of dose-response curves of the effect of TSH and HTS stimulation on cAMP increment showed an increase in the Km upon the addition of NaCl to the medium. A similar inhibitory effect of NaCl (150 mM) was also observed in the assay system of human thyroid adenylate cyclase stimulator using crude plasma membrane fractions. In summary: (1) an assay for HTS measuring cAMP production in cultured thyroid adenoma cells was developed, and the assay using low NaCl medium was found to be the most sensitive; and (2) the inhibitory effect of NaCl on the response to HTS was much greater than that on the response to TSH. These data suggest different behaviors of these two stimulators at their receptor sites. (Author abstract) (26 Refs)

CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES

13. PROSTAGLANDIN E2 AND CYCLIC AMP IN TUMOR AND PLASMA OF BREAST CANCER PATIENTS.

Malachi T, Chaimoff C, Feller N, Halbrecht I
(c/o Halbrecht) B. Gattegno Res. Inst., Hasharon Hosp., Petah-Tikva, Israel
J Cancer Res Clin Oncol; 102(1):71-79 1981

Prostaglandin E2 and cyclic AMP (cAMP) levels were measured in tumors and plasma of 78 patients with benign and malignant breast tumors. Two groups of malignant tissues were found, one with a high level of PGE2 (M = 55.4 pg/mg) and one with a low level (M = 10.7 pg/mg). The low level did not differ significantly from the benign tissue level (M = 8.7 pg/mg). Two malignant groups could not be detected in the plasma levels. Plasma PGE2 concentration (in form of the 13,14-dihydro-15-Keto metabolite) did not reflect the tissue levels, and no difference was found between the benign (M = 59.9 pg/ml) and the malignant (M = 62.3 pg/ml) patients, but both concentrations were higher than those of healthy controls (M = 34.4 pg/ml). The stage of the cancer, the histological classification and, most important, the period of survival, could not be related to the differences in the PGE2 tissue levels. Neither could plasma cAMP be nominated as a breast cancer marker because no difference was found between the cAMP levels of benign tumor patients (M = 16.48 pmol/ml), of malignant tumor patients (M = 21.24 pmol/ml) and of healthy controls (M = 19.07 pmol/ml). The conclusion is that although high amounts of PGE2 appear in some malignant breast tumors, they do not affect the clinical situation. These results may explain the failure to treat human breast cancer patients with prostaglandin synthetase inhibitors. (Author abstract) (28 Refs)

14. EFFECT OF THE RADIOPROTECTANT WR 2721 UPON CYCLIC NUCLEOTIDE LEVELS, LYSOSOMAL ENZYME ACTIVITIES, AND LYSOSOMAL MEMBRANE STABILITY (MEETING ABSTRACT).

Trocha PJ, Catravas GN
Armed Forces Radiobiology Research Institute, Bethesda, MD, 20014
Conference on Chemical Modification: Radiation and Cytotoxic Drugs, September 17-20, 1981, Key Biscayne, Florida. Committee for Radiation Oncology Studies 139 pp., 1981.

Biochemical effects of the radioprotectant S-2-(3-aminopropylamino)ethyl-phosphorothioic acid (WR 2721) were investigated in order to determine if this drug alters cyclic nucleotide levels and/or lysosomal systems. Using immunoassay and enzymatic techniques, liver and spleen cyclic nucleotide levels and lysosomal beta-glucuronidase activities were measured in irradiated and unirradiated rats injected ip with saline or WR 2721 dissolved in saline. No alterations in lysosomal beta-glucuronidase activities, lysosomal membrane stabilities, or cyclic nucleotide levels were found in the spleen or liver of unirradiated rats 1-6 hr after injection of the radioprotectant, except for spleen cyclic guanosine monophosphate (cGMP) content. Within 1 hr after administration of WR 2721, spleen cGMP levels were 50% lower than their normal concentration. However, 6 hr after injection of the radioprotectant, spleen cGMP levels were again normal. Animals treated with WR 2721 30-40 min prior to irradiation displayed a reduction in spleen lysosomal enzyme activities 1-3 days postirradiation compared with irradiated controls given only saline. Cyclic nucleotide levels were also affected by the radioprotectant. Liver and spleen cAMP levels were found to be higher 3-6 hr postirradiation; whereas, spleen cGMP levels were lower 1-4 hr postirradiation than the corresponding irradiated controls. At 3 days after radiation exposure, spleen lysosomal membrane stability and cyclic nucleotide levels in drug-treated rats were found to be closer to the levels of unirradiated control rats than of those of irradiated controls receiving saline. (no Refs)

15. THE REGULATION OF THE HUMAN ANTITUMOR IMMUNE RESPONSE TO ORGAN-SPECIFIC NEOANTIGENS.

Thomson DM, Phelan K, Scanzano R, Fink A, Bortin MM, Ferrone S, Martin WJ, Pious D
Montreal General Hosp. Res. Inst., Montreal General Hosp., 1650 Cedar Ave., Montreal, Quebec H3G 1A4, Canada
Transplant Proc; 13(4):1952-1954 1981

The mechanism of WBC adherence inhibition (LAI) is discussed. By increasing free intracellular Ca, Ca ionophore and ouabain stimulate a positive response. Lidocaine affects the binding of Ca to membranes and inhibits Ca++ flux; without Ca++ flux, antigen binding does not mediate LAI. Substances that increase intracellular cyclic AMP do not alter an LAI-positive response but convert to positive LAI-negative responses of patients with large tumor burdens. When prostaglandin E2 synthesis is blocked by indomethacin, the response of LAI-positive WBC is unaffected. When the generation of metabolities of the lipoxygenase pathway is blocked, the LAI-positive response is negated. Bridging of cytophilic antibod y receptors on membranes of monocytes by tumor antigens is probably transduced into information useful to the cell. The transduction is believed to involve the opening of Ca channels in the cell membrane. Metabolites of the lipoxygenase pathway play an important role in mediating the response to the tumor antigens. Control of the biochemical reaction activated by Ca entry seems to involve intracellular cyclic nucleotide levels. LAI in early cancers is discussed. (13 Refs)

16. PLASMA MEMBRANE-MEDIATED EFFECTS OF EXTRACELLULAR PH ON THE GROWTH OF NEUROBLASTOMA CELLS.

van der Saag PT, Feyen A, Miltenburg-Vonk W, De Laat SW

Hubrecht Lab., International Embryological Inst., 3584 CT Utrecht, The Netherlands

Exp Cell Res; 136(2):351-358 1981

Mouse neuroblastoma cells (Neuro-2A) were cultured at pH values between 6.5 and 8.0. Acidification of the culture medium resulted in a reversible inhibition of growth. To explore the possibility that the effect of extracellular pH on growth is mediated by the plasma membrane, plasma membrane microviscosity, cellular cAMP levels and adenylate cyclase activities were measured after exposing the cells to various pH levels. Upon lowering the extracellular pH from 7.6 to 6.5, plasma membrane microviscosity decreases within a few hr to 50%. Concomitantly cellular cAMP levels increase more than three-fold, while adenylate cyclase activity is enhanced three-fold as well, accompanied by a small increase in Km of the enzyme. All these effects are reversible. These results are compatible with the idea that the growth-regulatory role of the extracellular pH is mediated at the level of the plasma membrane, probably by altered membrane-adenylate cyclase interactions. (Author abstract) (65 Refs)

PROTEIN KINASE STUDIES

17. INTERACTION OF VASOACTIVE INTESTINAL PEPTIDE WITH HELA CELLS: ACTIVATION OF CYCLIC AMP-DEPENDENT PROTEIN KINASE AND LACK OF EFFECT ON DNA SYNTHESIS.

Prieto JC, Pintado E, De Miguel C, Guerrero JM, Goberna R

Catedra de Bioquimica, Facultad de Medicina, Alcala de Henares, Madrid, Spain

Biochem Biophys Res Commun; 103(3):799-805 1981

Protein kinase activity stimulated by vasoactive intestinal peptide (VIP) was studied in HeLa cells. Max activation required the simultaneous presence of a phosphodiesterase inhibitor. The response was dose dependent and in accordance with the concentration of VIP required for half-max stimulation of cyclic AMP production. Secretin stimulation of protein kinase and stimulation of DNA synthesis were also studied. (18 Refs)

18. ROUS SARCOMA VIRUS TRANSFORMING PROTEIN, P60SRC, EXPRESSED IN E. COLI, FUNCTIONS AS A PROTEIN KINASE.

Gilmer TM, Erikson RL

Dept. Pathology, Univ. Colorado Health Sciences Center, Denver, CO, 80262

Nature; 294(5843):771-773 1981

Rous sarcoma virus (RSV) p60src produced by Escherichia coli which carry plasmid s expressing the src gene was selected by immunoaffinity chromatography and tested for expression of protein kinase (PK) activity. The p60src virus phosphorylated tyrosine residues in tubulin and casein substrates; phosphorylation was inhibited by anti-p60src IgG in a reaction that resulted in phosphorylation of the immune IgG. No PK activity was detectable in bacterial preparations lacking the src gene. Expression of p60src-specific PK activity in E coli suggests that the enzyme activity observed in eukaryotic cells is encoded in the src gene. (25 Refs)

19. SIMILARITIES AND DIFFERENCES BETWEEN THE EFFECTS OF EPIDERMAL GROWTH FACTOR AND ROUS SARCOMA VIRUS.

Cooper JA, Hunter T

Tumor Virology Lab., Salk Inst., San Diego, CA, 92138

J Cell Biol; 91(3):878-883 1981

We have derived a line of A431 human tumor cells infected with Rous sarcoma virus (RSV). The infected cells contain the RSV-transforming protein, pp60src, which has characteristic tyrosine-specific protein kinase activity. As in other RSV-transformed cells, a 36,000-dalton protein is phosphorylated in RSV-infected A431 cells. Addition of epidermal growth factor (EGF) to the cells induces further phosphorylation of this protein. In contrast, this phosphoprotein is not detected in uninfected A431 cells, except when treated with EGF. Increased phosphorylation of the EGF receptor protein and of an 81,000-dalton cellular protein is dependent upon addition of EGF to the culture fluids, in both control and RSV-infected A431 cells. The results are discussed with reference to the similarities and differences between the tyrosine-specific protein kinases induced by RSV and activated by EGF. (Author abstract) (42 Refs)

20. RECENT DISCOVERIES ON CELLULAR PROTEINS IN RELATION TO VIRALLY AND CHEMICALLY INDUCED CARCINOGENESIS.

Jornvall H

Kemiska Institutionen I, Karolinska Institutet, S-104 01 Stockholm, Sweden

Lakartidningen; 78(41):3585-3586 1981

Recent studies on the molecular mechanisms of carcinogenesis suggest the existence of a cellular protein ('53K') common to virally and chemically induced cell transformations. This type of protein binds some T-antigens from oncogenic DNA viruses, including large T-antigen from SV40 and Epstein-Barr virus-determined nuclear antigen. Transformation-related proteins from oncogenic RNA viruses are similar to normal cellular proteins. Several of these proteins have phosphorylating activity, and some of them have been found to be protein kinases. Associations of such proteins with the plasma membrane have also been shown. These different connections between various tumor-related proteins and normal cellular proteins suggest the importance of gene determination in transformations. (11 Refs) (In SWE)

21. SYNTHETIC PEPTIDE FRAGMENT OF SRC GENE PRODUCT INHIBITS THE SRC PROTEIN KINASE AND CROSSREACTS IMMUNOLOGICALLY WITH AVIAN ONC KINASES AND CELLULAR PHOSPHOPROTEINS.

Wong TW, Goldberg AR
Rockefeller Univ., New York, NY, 10021
Proc Natl Acad Sci USA; 78(12):7412-7416 1981

All the known avian sarcoma viruses have associated protein kinase activities that phosphorylate tyrosine residues of their target proteins. A decapeptide fragment of pp60src of Rous sarcoma virus (RSV), residues 415-425, and an analog of that sequence have been chemically synthesized by solid-phase methods. The two decapeptides were not phosphorylated by pp60src of RSV, P90 of Y73 avian sarcoma virus, or P140 of Fujinami sarcoma virus. However, both peptides were able to inhibit competitively the kinase activities associated with the transforming proteins. Antiserum was raised against one of the peptides and IgG was purified from the serum by affinity chromatography. The antibody was able to precipitate pp60src of RSV as well as P90 of Y73 virus from cells infected with these viruses. The antibody also precipitated a number of high molecular weight phosphoproteins from normal chicken and rat fibroblasts and from several lines of virus-transformed cells. (Author abstract) (29 Refs)

22. GENE PRODUCTS OF MCDONOUGH FELINE SARCOMA VIRUS HAVE AN IN VITRO-ASSOCIATED PROTEIN KINASE THAT PHOSPHORYLATES TYROSINE RESIDUES: LACK OF DETECTION OF THIS ENZYMATIC ACTIVITY IN VIVO.

Barbacid M, Lauver AV
Lab. Cellular and Molecular Biology, NCI, Bethesda, MD, 20205
J Virol; 40(3):812-821 1981

The primary translational product of the McDonough (SM) strain of feline sarcoma virus (FeSV) is a 180,000-dalton molecule, SM P180, that contains the p15-p12-p30 region of the FeLV gag gene-coded precursor protein and a sarcoma virus-specific polypeptide. In addition, cells transformed by SM-FeSV express a 120,000-dalton molecule, SM P120, that is highly related to the non-helper virus domain of SM P180. Both SM-FeSV gene products were found to be intimately associated with the membrane fraction of SM-FeSV-transformed cells. Immunoprecipitates containing SM P180 and SM P120 exhibited a protein kinase activity capable of phosphorylating tyrosine residues of both viral gene products but not immune immunoglobulin G molecules. By independently immunoprecipitating each of the two SM-FeSV proteins we found that most of the tyrosine-specific phosphorylating activity was associated with the SM P120 molecule. In vivo analysis of 32P-labeled SM P180 and SM P120 revealed their phosphoprotein nature; however, both molecules exhibited low levels of phosphorylation and did not contain phosphotyrosine residues. Finally, we did not detect any significant elevation in the levels of phosphotyrosine in the protein fraction of SM-FeSV transformants. Thus, if SM-FeSV were to induce malignant transformation by a mechanism involving phosphorylation of tyrosine residues, the viral gene products must interact with a small subset of cellular proteins that do not represent a significant fraction of the total cellular protein content. (Author abstract) (44 Refs)

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AUTHOR INDEX WITH CITATION NUMBERS

Amr S 9
Anderson WB 2
Barbacid M 22
Binet E 2
Black PM 1
Bortin MM 15
Carayon P 9
Catravas GN 14
Chaimoff C 13
Cooper JA 19
Criss WE 7
Davison PF 1
De Laat SW 16
De Miguel C 17
Erikson RL 18
Evain D 2
Feller N 13
Ferrone S 15
Feyen A 16
Findlay DM 5
Fink A 15
Gilmer TM 18
Goberna R 17
Goldberg AR 21
Guerrero JM 17
Gunther GR 6
Halbrecht I 13
Hung P 3
Hunter T 19
Iida Y 12
Ikekubo K 12
Ishibashi S 8
Jornvall H 20
Jungmann RA 3
Kasagi K 12
Kitamura Y 8
Konishi J 12
Kornblith PL 1
Kuma K 12
Kurokawa T 8
Lamp SJ 5
Lauver AV 22
Liszczak TM 1
Majeska RJ 4
Malachi T 13
Martin TJ 5
Martin WJ 15
McKeever PE 1
Merk LP 1
Miles MF 3
Miltenburg-Vonk 16
Mori T 12
Moriuchi M 8
Morris HP 7
Moseley JM 5
Pekonen F 9
Phelan K 15
Pintado E 17
Pious D 15
Prieto JC 17
Quindlen EA 1
Rochette-Egly C 10
Rodan GA 4
Scanzano R 15
Slaughter L 7
Smith BH 1
Thomson DM 15
Torizuka K 12
Tovey MG 10
Tovey MG 11
Trocha PJ 14
Van der Saag PT 16
Weintraub BD 9
Wong TW 21

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In this issue:

- REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES 1-7
- REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES 8-12
- CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES 13-14
- CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES 15-16
- PROTEIN KINASE STUDIES 17-28

REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES

1. A MORPHOLOGIC STUDY OF THE DISTRIBUTION OF CYCLIC AMP AND CYCLIC GMP IN PERIPHERAL AND BONE MARROW CELLS OF NORMAL AND LEUKEMIC SUBJECTS BY THE PEROXIDASE-ANTIPEROXIDASE PAP METHOD.

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Haematologica (Pavia); 66(5):588-596 1981

The peroxidase-antiperoxidase (PAP) method was used to study the distribution of cyclic AMP (cAMP) and cyclic GMP (cGMP) in peripheral and bone marrow cells of normal subjects and leukemic patients. The leukocytes and lymphoblasts of patients with chronic myelogenous leukemia and acute lymphocytic leukemia showed no degree of positive cAMP reaction to the PAP test as compared to the normal degree of positive reaction of nonleukemic subjects. However, positive cGMP reactions (as great as 4x normal) were observed in the leukemia cells. the cGMP distribution was almost exclusively cytoplasmic. Immunofluorescence and immunoperoxidase were also used to test for cAMP an cGMP but no results were obtained. (5 Refs) (In ITA)

2. THE PRIMARY TISSUE CULTURE OF HUMAN ADRENOCORTICAL CONN'S ADENOMATA. I. THE SYNERGISTIC STIMULATION OF ADENOMATOUS CELL GROWTH BY PURINE CYCLIC NUCLEOTIDES AND BY ACTH1-24 AND ANGIOTENSIN II.

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Pathol Res Pract; 173(1/2):66-81 1981

Primary cultures of dissociated parenchymal cells from Conn's adenomata causing primary hyperaldosteronism were successfully set up by a method previously used with normal adult human and rat adrenocortical tissue. In such cultures the adenomatous cells largely prevailed (making up 87% of the whole cell population), could survive for lengthy terms (at least up to 30 days), and were endowed with a spontaneous, discrete capability to proliferate. The de novo RNA- and DNA-synthetic and mitotic activities of Conn's cells were markedly stimulated in cultures exposed between day 16 and 21 to daily doses of exogenous cyclic AMP, either alone or in equimolar association with cyclic GMP. A significantly weaker, though still prominent enhancement of adenomatous cell growth was elicited also by daily administrations of an equimolar mixture of ACTH1-24 and angiotensin II. In contrast, little stimulation or inhibition of growth or no effect at all could be observed when cyclic GMP, ACTH1-24, and angiotensin II were respectively administered, each by itself. The present results support the working hypothesis that Conn's adenomatous cell growth is orderly controlled by several hormonal factors, including angiotensin and ACTH, the actions of which are mediated by both cyclic AMP and GMP. (Author abstract) (39 Refs)

3. WHEN YOU SUSPECT HYPERCALCEMIA.

Brown EM
(c/o Editor), 16 Thorndal Circle, Darien, CT, 06820
Patient Care; 16(2):14-18,25-27,30-32,37 1982

Evaluation and treatment of suspected hypercalcemia are discussed. Physical examination, history (especially drug history), and several laboratory tests can usually distinguish between hyperparathyroid hypercalcemia and other forms of hypercalcemia not mediated by parathyroid hormone (PTH). In humoral, malignancy-associated hypercalcemia, urinary cyclic AMP levels are elevated. A complete blood count and RBC sedimentation test may disclose multiple myeloma or another hematologic malignancy. In hypercalcemia not associated with excess PTH, bone scans and skeletal x-rays may disclose a bone malignancy. Even if the patient has a malignancy (eg, breast cancer with bone metastases), the associated hypercalcemia might be caused by primary hyperparathyroidism or another benign condition. Review of the patient's previous laboratory tests may disclose evidence of chronic hypercalcemia. The most important factor in treatment of hypercalcemia is treatment of the underlying disease. Therapy designed to increase renal calcium excretion and reduce bone resorption include a short course of mithramycin (15-25 ug/kg) and a combination of glucocorticoids and calcitonin. At least in treatment of malignancy-associated hypercalcemia, the glucocorticoid-calcitonin combination can maintain nearly normal levels of serum calcium for 4-5 days longer than calcitonin alone. (3 Refs)

4. COEXISTENT MULTIPLE MYELOMA AND PRIMARY HYPERPARATHYROIDISM.

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JAMA; 247(6):823-824 1982

A patient with multiple myeloma and hypercalcemia responded to cytotoxic chemotherapy. However, hypercalcemia persisted. Because of the absence of lytic bone lesions, the presence of a low serum phosphate level, and a family history of possible primary hyperparathyroidism, the patient was evaluated for this disorder. Serum parathyroid hormone and urinary cyclic adenosine monophosphate levels were elevated. Exploration of the neck disclosed two enlarged parathyroid glands (1,850 mg and 210 mg), which were excised. After surgery, the patient's serum calcium levels remained normal for one year. Progressive myeloma bone disease developed that eventually resulted in recurrent hypercalcemia and death. Autopsy revealed only evidence of myeloma. (Author abstract) (9 Refs)

5. CYCLIC AMP-RECEPTOR PROTEINS IN HUMAN MCF-7 CELLS AND BREAST CANCER (MEETING ABSTRACT).

Eppenberger U, Almendral A, Huber P, Kung W, Handschin J

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UICC Conference on Clinical Oncology, October 28-31, 1981, Lausanne, Switzerland. International Union Against Cancer 131 pp., 1981.

Several recent investigations have indicated that a relationship exists between intracellular levels of cyclic (c)AMP and cAMP-dependent protein kinase isozymes type I and type II during normal and neoplastic growth and tumor regression. This study demonstrates a significant alteration in the cAMP-receptor protein pattern (regulatory subunit R-II of type II) of human neoplastic mammary tissue from surgical biopsies and human mammary MCF-7 cells as compared to normal and dysplastic mammary tissues. Photoaffinity labeling of the cAMP receptors with 8-N3-[32P] cAMP revealed on sodium dodecyl sulfate polyacrylamide gels 3 cAMP-receptor proteins of R-II (Mol wt (MW) 56,000, 54,000 and 52,000) and cAMP-receptor protein with an apparent MW of 40,000, which could not be detected in normal and dysplastic mammary cells. It is suggested that the 40,000 MW receptor results from proteolysis and has some prognostic value with respect to endocrine therapy and DBcAMP treatment of breast cancer (IND No 2025) which is under investigation. Only small amounts of the regulatory subunit R-I of isozyme type I were observed in MCF-7 cells and breast cancer tissue, whereas in normal mammary tissue no R-I could be found. (no Refs)

6. POSSIBLE EFFECTIVENESS OF ADDITION OF P-AMINO-BENZOIC ACID-N-XYLOSIDE ADMINISTRATION TO IRRADIATION (MEETING ABSTRACT).

Fukushima M, Ota K

Department of Internal Medicine, Aichi Cancer Center, Nagoya, Japan

Proceedings of the Japanese Cancer Association, 40th Annual Meeting, October 1981, Sapporo, Japan. The Association 415 pp., 1981.

P-Aminobenzoic acid-N-xyloside (K-247) is an agent recently introduced that affects cell membrane and elevates intracellular concentration of prostacyclin (PGI2), prostaglandin D2 (PGD2), and cyclicAMP. It is reported that the compound has an antitumor activity and enhances the effect of mitomycin C or cyclophosphamide. Noguchi et al reported that the combination of irradiation (XRT) and K-247 was more effective than single therapy in Sarcoma-180. We report here a preliminary clinical trial of combination therapy of XRT with K-247. The cases which were considered to be relatively resistant to XRT alone had received 800 mg of K-247 daily during XRT (2000 to 3000 rads). Marked responses to the combination therapy were observed in four cases including tumor of mediastinum and neck (leiomyoma), metastatic supraclavicular tumor from duodenal carcinoma (adeno ca), lung carcinoma associated with carcinomatous pleuritis (epidermoid ca), and middle pharyngeal ca with tongue invasion and neck metastasis (squamous cell ca). This result suggests that K-247, a kind of biological response modifier, seems to enhance the effect of XRT. (no Refs)

7. ADJUVANT BCG TUMOUR ANTIGEN FOLLOWING PRIMARY TUMOUR RESECTION: RESULT OF MELBOURNE UNIVERSITY 5 YEAR STUDY (MEETING ABSTRACT).

Steward AM, Clarebrough JK, Wilson A

Departments of Surgery and Cardiothoracic Surgery, University of Melbourne, Melbourne, Australia

Clinical Oncology Society of Australia, November 24-27, 1981, Melbourne, Australia. The Society 70 pp., 1981.

Thirty-four lung cancer patients have been entered by random assignment into a trial of adjuvant therapy using BCG (skin scarification) +/- autologous tumor antigenic preparation (subcutaneous innoculation), following resection of their primary tumor. All treated patients received 3-7 BCG doses at 2-4 weekly intervals, beginning 1 mo following operation. Six also received antigenic tumor preparation by hypertonic KCl fractionation. The treated group consisted of 11 Squamous, 3 Large Cell, 1 Adeno, and 2 Small Cell carcinomas. Controls were 7 Squamous, 6 Large Cell, 2 Giant Cell, 1 Adeno and 1 Small Cell. In the treated group, 7 out of 10 stage I and II are alive and well at 37-62 mo, compared with 2 of 11 controls plus 1 with metastases. Mean survival of those dying in the treated group was 30 mo, compared with 12.8 mo (control). In monitoring patient progress, serial plasma cyclic AMP (c-AMP) levels, serum T3 and thyroid stimulating hormone (TSH) were estimated in 5 patients from each group. Five patients who died within 15 mo, had c-AMP values of 1.1-9.2 Pmol/ml and 4 of these had falling T3 values. Five patients who remained alive and disease free at 16-31 mo had c-AMP values of 0.7-24.8 Pmol/ml, and static or rising T3 levels. Adjuvant therapy given to the treated group prolonged survival, disease free intervals, and appeared to improve quality of life. (no Refs)

REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES

8. CYCLIC AMP-BINDING PROTEIN AND ESTROGEN RECEPTOR: ANTAGONISM DURING NUCLEAR TRANSLOCATION IN A HORMONE-DEPENDENT MAMMARY TUMOR.

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Biochem Biophys Res Commun; 103(4):1349-1355 1981

Mutual inhibition of translocations from the cytoplasm to the nucleus by estrogen receptor (ER) and cyclic AMP-binding protein was examined in a cell-free system of mammary tumors. Incubation of nuclei from hormone-dependent MTW9 rat mammary tumors with cytosol activated by 5 nano (n)M 17beta-estradiol resulted in a 4-fold increase in nuclear estrogen binding activity over the control nuclei. The presence of 100 nM cyclic AMP (cAMP) in the activated cytosol inhibited nuclear uptake of ER by 50%. Incubation of the nuclei with cytosol activated by 100 nM cAMP increased nuclear cAMP binding and cAMP-dependent protein kinase activity 4-fold. The presence of 5 nM 17beta-estradiol in the activated cytosol inhibited the nuclear cAMP binding and the protein kinase activity by 50%. No competition was found between estrogen and cAMP for each other's cytoplasmic binding proteins or the nuclear acceptor sites. Data suggest that a mutual antagonism exists between the cAMP-binding protein and ER during their nuclear translocation; the mutual antagonism was dependent on concentrations of the ligands in the activated cytosol. Activated glucocorticoid receptors did not influence the nuclear uptake of either ER or cAMP-binding proteins. Studies on the structural conformation of cAMP- and estrogen binding proteins under controlled conditions of phosphorylation and dephosphorylation may elucidate the mechanism of antagonism between the receptors. (15 Refs)

9. TWO DISTINCT INTRACELLULAR PATHWAYS TRANSPORT SECRETORY AND MEMBRANE GLYCOPROTEINS TO THE SURFACE OF PITUITARY TUMOR CELLS.

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Cell; 28(1):51-59 1982

The pituitary cell line, AtT-20, synthesizes adrenocorticotropic hormone (ACTH) as a glycoprotein precursor that is cleaved into mature hormones during packaging into secretory granules. The cells also produce an endogenous leukemia virus (MuLV) that is glycosylated after translation similar to the glycosylation of the ACTH precursor. Our evidence suggests that the envelope glycoprotein and some precursor ACTH get to the cell surface in a vesicle different from the mature ACTH secretory granule. Viral glycoproteins and ACTH precursor are released from the cells much sooner after synthesis than mature ACTH. Isolated secretory granules do not contain significant amounts of the envelope glycoprotein or ACTH precursor. Exposing cells to 8Br-cAMP stimulates release of mature ACTH four to five fold, but has little effect on the release of the ACTH precursor or the viral glycoproteins. We propose that the viral glycoproteins and some of the ACTH precursor are transported by a constitutive pathway, while mature ACTH is stored in secretory granules where its release is enhanced by stimulation. (Author abstract) (32 Refs)

10. STIMULATION OF ALBUMIN SYNTHESIS IN MOUSE HEPATOMA CELLS BY BT2CAMP: CELL CYCLE SPECIFICITY.

Hamman HC, Ledford BE
Dept. Pharmacology, Univ. Virginia, Charlottesville, VA, 22908
Arch Biochem Biophys; 213(2):620-624 1982

Effects of cyclic AMP on mouse hepatoma cells (Hepa) proliferation and on albumin synthesis occurring during a specific phase of the cell cycle were studied. Relative rates of albumin synthesis were measured at various times after addition of dibutyryl cyclic AMP (Bt2cAMP) to the growth medium of synchronously growing cells. Results indicate that the relative rate of albumin synthesis increases after the cells enter the G1 phase of the cycle and that Bt2cAMP is required during the S phase. (15 Refs)

11. REGULATION OF AFLATOXIN BIOSYNTHESIS: EFFECT OF EXOGENOUSLY SUPPLIED CYCLIC NUCLEOTIDES.

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J Food Sci; 47(1):153-157 1982

The effects of cAMP, cGMP, AMP, and GMP on aflatoxin accumulation were studied using flask and test tube cultures of Aspergillus parasiticus. The response to cGMP supplementation was variable, depending on the type of culture system employed. Cyclic AMP produced a dose-related increase in aflatoxin production, with 5.0 mM supplementation resulting in as much as an 18-fold increase in toxin accumulation. Stimulation of aflatoxin production by cAMP was not attributable to nutritional effects, and the results suggest that cAMP may be a key regulatory control point governing aflatoxin biosynthesis. (Author abstract) (17 Refs)

12. DISTINCTION BETWEEN PARANEOPLASTIC HYPERCALCEMIA (HCA) AND HYPERCALCEMIA OF PRIM ARY HYPERPARATHYROIDISM (PHP) BY MEANS OF BIOCHEMICAL PARAMETERS (MEETING ABSTRACT).

Burckhardt P, Bill G, Jacquet AF
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UICC Conference on Clinical Oncology, October 28-31, 1981, Lausanne, Switzerland. International Union Against Cancer 131 pp., 1981.

HCa is the most frequent metabolic complication of malignant disease. Its major differential diagnosis is PHP. This often relies on biochemical parameters of parathyroid function, such as 1) plasma parathyroid hormone (PTH), 2) chloremia/phosphoremia ratio (Cl/P), 3) nephrogenous cyclic AMP (NcAMP). We studied the diagnostic reliability of these 3 parameters: in 57 patients with HCa, PTH was found to be suppressed in 12, low normal in 33, high normal in 10, and elevated in 2. Only in these 2 patients paraneoplastic secretion of PTH could be suspected. In 45 patients with PHP, PTH was low normal in 2, high normal in 12, and elevated in 31. The overlap between the 2 groups occurred in mild hypercalcemia only, but not with a plasma calcium of over 3 mmol/1. 2) The Cl/P was measured in 29 patients with HCa and 29 patients with PHP. Five patients with HCa and all patients with PHP had values above normal, but there was no overlap between the 2 groups, except of one single case with HCa. 3) The NcAMP was normal or low in all 10 patients with HCa studied, and above normal in 9 out of 10 patients with PHP, without overlap. Conclusion: with none of the 3 parameters suppressed PTH secretion could be documented in all patients with HCa. As routine analysis Cl/P is the most practical test for an almost complete distinction between the 2 causes of hypercalcemia. Complete separation was obtained by NcAMP, and by PTH when hypercalcemia exceeded 3 mmol/1. (no Refs)

CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES

13. TUFTSIN, THR-LYS-PRO-ARG: ANATOMY OF AN IMMUNOLOGICALLY ACTIVE PEPTIDE.

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Mol Cell Biochem; 41:73-97 1981

Tuftsin, a natural occurring tetrapeptide, has been found to exhibit several biological activities connected with immune system function. Although little is known about tuftsin's biogenesis, much information has been gleaned about its structure-function relationships, which have shown that several features of the molecule are essential for expression of full biological activity. Furthermore, specific receptor sites for tuftsin have been found to exist exclusively on phagocytic cells. Research indicates that tuftsin binding to target cells affects intracellular calcium and cyclic nucleotide levels. Implication of these facts on tuftsin's mode of action are discussed. Basic peptide segments resembling tuftsin are found in a variety of regulatory peptides. Questions are, therefore, raised as to the biospecificity and cross-reactivity of these sequences. Substance P, one such peptide, which binds with and activates tuftsin receptors, is described. In light of tuftsin's therapeutic potential, assays for its determination have been introduced. When applied to the analysis of human blood serum of normal as well as of various pathological origins, direct correlation was found between tuftsin levels and susceptibility to bacterial infections. (Author abstract) (116 Refs)

14. RNA POLYMERASE II IN C6 GLIOMA CELLS. ALPHA-AMANITIN BLOCKADE OF CAMP PHOSPHODIESTERASE INDUCTION BY BETA-ADRENERGIC STIMULATION.

Schwartz JP
Lab. Preclinical Pharmacology, Natl. Inst. Mental Health, Saint Elizabeth's Hosp., Washington, DC, 20032
Exp Cell Res; 137(1):39-45 1982

C6 glioma cells contain an RNA polymerase activity which is nuclear, requires Mn++ and high ionic strength for maximal activity, is inhibited by low concentrations of alpha-amanitin, or by actinomycin D, and is therefore classified as RNA polymerase II. Exposure of intact cells to isoproterenol, a beta-adrenergic receptor agonist, results in an increase in the activity of cAMP phosphodiesterase. This increase in phosphodiesterase activity is blocked by incubation of the cells with alpha-amanitin or actinomycin D, suggesting that RNA polymerase II is necessary for synthesis of mRNA for the increase in phosphodiesterase activity. RNA polymerase II activity is not increased in isoproterenol-treated cells, although it is more strongly inhibited by alpha-amanitin. The Ki for the effect of alpha-amanitin on intact cells is 250 nM, and the inhibitor acts rapidly, with a maximal effect within 15 min of exposure. The effect of alpha-amanitin is very specific, as no change in various cytoplasmic or nuclear enzymes or metabolites was observed. (Author abstract) (21 Refs)

CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES

15. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR IDENTIFICATION AND QUANTITATION OF NUCLEOTIDES IN LYMPHOCYTES AND MALIGNANT LYMPHOBLASTS.

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Div. Pediatric Oncology, St. Radboud Hosp., Catholic Univ. Nijmegen, Nijmegen, The Netherlands

J Chromatogr; 227(1):45-52 1982

A method for the identification and quantitation of nucleotide pools in lymphocytes and leukemic blasts is described. Separation of these metabolites was performed by anion-exchange high-performance liquid chromatography using a pH and concentration gradient consisting of several linear steps. The mono-, di- and triphosphates of adenosine, cytidine, guanosine, inosine, uridine and xanthosine could conveniently be separated together with NAD+, cyclic AMP, NADP+ and uridinediphosphoglucose (UDPG). In addition, data on the accuracy and precision of the method are given and its potentials for use in the analysis of nucleotide pools in leukemic lymphoblasts are illustrated. (Author abstract) (15 Refs)

16. INHIBITION OF CARDIAC GUANYLATE CYCLASE BY DOXORUBICIN AND SOME OF ITS ANALOGS: POSSIBLE RELATIONSHIP TO CARDIOTOXICITY.

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Cancer Treat Rep; 66(2):311-316 1982

The anthracycline antibiotic doxorubicin induces a variety of cardiotoxic effects. We have recently demonstrated that this drug also causes a selective inhibition of rat and human cardiac guanylate cyclase activity in vitro. In the present study, we examined the effect of 30 analogs of doxorubicin on cardiac guanylate cyclase activity. Structural modifications of these anthracycline antibiotics were found to alter their effect on rat cardiac guanylate cyclase activity. N-substitutions on the sugar moiety eliminated the inhibitory action observed with the parent compound. Long-chain hydrocarbon substitutions in place of the methylketone side chain had a similar effect. Removal or substitution of the C-4 methoxy group had little or no effect on the ability of these compounds to modify guanylate cyclase activity. Substitutions of the C-9 side chain by a hydrazone derivative resulted in compounds that stimulated the enzyme. All of the anthracenedione derivatives were inhibitory. A comparison of the inhibitory effect of some of these anthracycline derivatives on in vitro cardiac guanylate cyclase activity with their cardiotoxic potency suggests a possible relationship between these two parameters. (Author abstract) (43 Refs)

PROTEIN KINASE STUDIES

17. CAMP RECEPTOR PROTEINS AND PROTEIN KINASES IN HUMAN LYMPHOCYTES: FUNDAMENTAL ALTERATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS.

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Eur J Biochem; 120(3):585-592 1981

To study the significance of cAMP receptor proteins and protein kinase activities in tumor dys-differentiation, peripheral blood lymphocytes from normal donors and from patients with chronic lymphocytic leukemia were isolated by Percoll gradient centrifugation, and analyzed. Both normal and tumor lymphocyte populations were devoid of cell proliferation. Normal human lymphocytes contained about equal activities of histone kinase and 'casein kinase'. The tumor lymphocytes, however, exhibited drastically reduced values of all three protein kinase activities, the most pronounced decrease being observed with the 'casein kinase'. Total high-affinity cAMP-binding sites (= regulatory subunits R of cAMP-dependent protein kinases Type I and II) in leukemic lymphocytes were also strongly reduced. The findings in lymphocytes of chronic lymphocytic leukemia cannot be explained by the fact that they are B-type lymphocytes. Control experiments revealed that normal B cells do not differ significantly from total blood lymphocytes with respect to the parameters mentioned above. Biochemical correlates of the dys-differentiated status of these non-proliferating cells from patients with chronic lymphocytic leukemia, then, are represented by reduced levels of protein kinases, of basal cAMP as well as by changes in the R-protein pattern. The data indicate that both protein kinases Type I and Type II may relate to the state of differentiation, independently from cell proliferation. (57 Refs)

18. CONTRASTING PATTERNS OF PROTEIN KINASE ACTIVITIES AMONG NORMAL PERIPHERAL BLOOD LYMPHOCYTE SUBPOPULATIONS AND LYMPHOID CELL LINES.

Elias L, Longmire J, Bankhurst AD

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J Lab Clin Med; 99(1):56-63 1982

Human peripheral mononuclear cell suspensions were separated into E-rosette-positive (T cell) and E-rosette-negative (non-T cell) fractions and assayed for cAMP binding activity and histone and casein kinase activity, in the presence and absence of cAMP. Each of these activities was higher in E-rosette-positive than E-rosette-negative fractions. Cyclic AMP binding and histone kinase activities were further noted to be higher in T lymphoblastoid cell lines when compared to lines of non-T lymphoid cell origin. In addition, DEAE-cellulose chromatography revealed a qualitative contrast of isoenzymic composition of protein kinase from these cell lines. Cyclic AMP-independent casein kinase activity was high in both types of cell lines. These results suggest that high protein kinase activity is characteristic of T cells and may be related to their higher sensitivity to cAMP agonists in comparison to non-T cells. This study also provides further evidence for an association between increased cAMP-independent protein kinase activity and the transformed state, which has been noted for a variety of other cell types. (Author abstract) (30 Refs)

19. EFFECT OF RETINOIC ACID TREATMENT OF F9 EMBRYONAL CARCINOMA CELLS ON THE ACTIVITY AND DISTRIBUTION OF CYCLIC AMP-DEPENDENT PROTEIN KINASE.

Plet A, Evain D, Anderson WB
Unite Institut National de la Sante et de la Recherche Medicale 188, 75014 Paris, France
J Biol Chem; 257(2):889-893 1982

Treatment of F9 teratocarcinoma cells with all trans-retinoic acid previously has been shown to induce differentiation to parietal endoderm and to enhance the responsiveness of these cells to the addition of cyclic AMP. Evidence is presented that retinoic acid treatment of F9 cells causes an increase in both cytosolic- and plasma membrane-associated cyclic AMP-dependent protein kinase activities. Retinoic acid elicits a progressive increase in cytosolic kinase activity through 72 hr. Cyclic AMP-dependent kinase activity of the plasma membrane fraction is enhanced within 3 hr of retinoid treatment of F9 cells and reaches a maximum at 15 hr after the addition of retinoic acid. The amount of regulatory subunits of cyclic AMP-dependent protein kinases I (RI) and II (RII) present in the subcellular fractions was quantitated by photoaffinity labeling with 8-azido-cyclic [32P]AMP. In the cytosol, the amount of RI and RII both increase with the period of retinoid treatment through 24 hr, and remain elevated through 5 days; the ratio of RI/RII (approx 2.5) found in the cytosol remains constant following retinoic acid treatment. RI is much more prevalent than RII (RI/RII = 7.5) in the plasma membrane prepared from untreated F9 cells. Retinoic acid treatment elicits a preferential increase in the amount of the RII regulatory subunit associated with the membrane fraction. After 15 hr of retinoid treatment, a 1.6-fold increase in RI is observed, while membrane-associated RII is increased 4.5-fold. After 24 hr of retinoic acid treatment, the level of RI returns toward the level noted with untreated cells, while the amount of RII remains significantly elevated through 5 days of retinoid treatment. These findings suggest that the increase in cyclic AMP-dependent protein kinase activity and the specific accumulation of RII associated with the plasma membrane fraction may be early events of retinoic acid action to mediate eventual cellular differentiation. (Author abstract) (38 Refs)

20. DIFFERENTIATION-SPECIFIC INCREASE OF CAMP-DEPENDENT PROTEIN KINASE IN THE 3T3-L1 CELLS.

Liu AY
Dept. Pharmacology, Harvard Medical Sch., Boston, MA, 02115
J Biol Chem; 257(1):298-306 1982

The specific activity of cAMP-dependent protein kinase was studied during the differentiation of 3T3-L1 fibroblasts into adipocytes. 8-Azido-adenosine 3':5'-monophosphate, a photoaffinity-labeling analogue of cAMP, was used to quantitate the regulatory subunits of the Type I and Type II cAMP-dependent protein kinase in cell extracts. The catalytic subunit of cAMP-dependent protein kinase was studied by assaying for cAMP-dependent histone kinase activity. A significant increase in cAMP-dependent protein kinase activity was observed during the adipose development of 3T3-L1 cells, promoted by 3-isobutyl-1-methylxanthine (IBMX), dexamethasone, and insulin. This increase was attributed primarily to a three- to six-fold increase in the Type I cAMP-dependent protein kinase, as demonstrated either by the incorporation of 8-azido-adenosine 3':5'-monophosphate into the 47,000-dalton regulatory subunit of the Type I kinase or by the separation and analysis of the Type I and Type II kinases through DEAE-Sephacel column chromatography. The time course of increase in the Type I cAMP-dependent protein kinase preceded increases both in malic enzyme and glutamine synthetase activities and was maximal at 5 days after initiation of the hormone treatment. The induction of cAMP-dependent protein kinase does not appear to involve an increase in intracellular cAMP. The addition of either 1 mM dibutyryl cAMP or 0.5 mM IBMX had little or no effect on the expression of cAMP-dependent protein kinase activity in the 3T3-L1 cells, although a marked increase in cAMP phosphodiesterase activity was observed in cells treated with dibutyryl cAMP. The increased expression of the Type I cAMP-dependent protein kinase in 3T3-L1 cells appears to be determined by the operation of the intrinsic differentiation program in these cells; it was observed in both the hormone-induced as well as spontaneously differentiated 3T3-L1 cells but was not observed in a control clone of 3T3, the 3T3-C2 cells, treated with IBMX-dexamethasone and insulin. These results suggest that the 3T3-L1 cells may provide an ideal system for studying the mechanism of regulation of cAMP-dependent protein kinase and its functional significance during development and differentiation. (Author abstract) (45 Refs)

21. PROTEIN KINASE ACTIVITY AND SUBSTRATES AT THE SURFACE OF INTACT HELA CELLS.

Kubler D, Pyerin W, Kinzel V
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J Biol Chem; 257(1):322-329 1982

Evidence is presented for the location at the surface of HeLa cells of a protein kinase capable of phosphorylating surface as well as extracellular (foreign) proteins. The reaction products have been found to be proteins containing phosphoryl groups as monoesters of seryl and threonyl residues (but not of tyrosine). The enzyme is of the cyclic AMP-independent type, since neither cyclic AMP nor the heat- and acid-stable inhibitor protein (specific for cyclic AMP-dependent protein kinases) influenced its activity. Further, co-substrate ATP could in part be substituted by GTP, and the spectrum of proteins phosphorylated by the ecto-enzyme differed from that phosphorylated by cyclic AMP-dependent protein kinases. Evidence for the ecto-enzymic nature of this protein kinase includes (a) utilization of co-substrate and location of products at the surface cells carefully controlled as being in an intact state and (b) phosphorylation of exogenous protein (phosvitin; specific serum proteins) by intact cells. Conclusive proof was gained by qualitative and quantitative comparative studies of phosphorylation in cultures with varying degrees of damaged cells either as a whole or after separation into groups of intact and damaged cells by electronic cell sorting. The results of experiments with cell sonicates excluded the possibility that either enzyme or substrates released from damaged cells were simply adsorbing to the cell surface. (Author abstract) (47 Refs)

22. BIOCHEMICAL CHARACTERIZATION OF PROTEIN KINASE ACTIVITIES ASSOCIATED WITH TRANSFORMING GENE PRODUCTS OF THE SNYDER-THEILEN AND GARDNER-ARNSTEIN STRAINS OF FELINE SARCOMA VIRUS.

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Lab. Viral Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY, 10021
Virology; 117(1):165-172 1982

Translation products of the genomes of the Snyder-Theilen (ST) and Gardner-Arnstein (GA) feline sarcoma viruses (FeSV) have been shown to possess associated protein kinase activities. These activities are similar to those associated with a number of rapidly transforming retroviruses in that they appear to mediate transfer of radiolabel from [gamma-32P]ATP to selected tyrosine residues in substrates and to be independent of the effects of cAMP and cGMP in vitro. The FeSV activities, when detected in specific immune complexes bound to Staphylococcus aureus, result predominantly in autophosphorylation of FeSV polyproteins. Partially purified polyprotein also induces phosphorylation of an exogenous acceptor (casein) in a soluble protein kinase assay system. The FeSV enzymes display marked preference for Mn++ and Co++ over Mg++ while Ca++ fails to initiate activity in vitro. The optimal pH for the reactions is near 7.0. There is a strict requirement for ATP as phosphoryl donor. High concentrations of ADP and dADP are inhibitory to the reaction but low concentrations of dADP are stimulatory. A comparison of these properties with those of other retroviral-associated protein kinases suggests that these activities fall into three, and possibly four, classes. (Author abstract) (52 Refs)

23. STUDIES ON PROTEIN KINASES IN TWO HUMAN RECTOCOLIC CELL LINES BY POLYACRYLAMIDE GEL ELECTROPHORESIS. EFFECT OF THE VASOACTIVE INTESTINAL PEPTIDE.

Mangeat P, Ait-Ahmed O, Marchis-Mouren G, Marvaldi J
(c/o Marchis-Mouren) Institut de Chimie Biologique, CNRS LA 202, 3 Place Victor-Hugo, 13331 Marseille Cedex 3, France
Biochim Biophys Acta; 714(2):320-330 1982

Electrophoresis and subsequent assay of the enzyme directly onto the gel has allowed a rapid and quantitative characterization of the cyclic AMP-dependent and -independent histone kinases, protamine, phosvitin and casein kinases in HT 29 and HRT 18 cells. The technique has been applied to soluble extracts from cytoplasmic and nuclear fraction prepared in the presence and absence of neutral detergent. A more precise identification of these enzymes has been possible by analyzing enzyme fractions obtained after ion-exchange chromatography of the above extracts. The protein kinase equipment of both cell lines was found to be identical (11 major components) but with different relative proportions of several enzymes. In cytoplasmic extracts: VIP activates only the Type I, cytosolic, (Band 4) and the Type II, membrane-bound, (Bands 6 and 8) cyclic AMP-dependent histone kinases. These enzymes account, respectively, for 34 and 55% of the total histone kinases in HT 29 and HRT 18 cells. The cyclic AMP-independent histone kinases (Band 1, 2, 5 and 7) also phosphorylate protamine; Band 5 was found to be much higher (4-fold) in HT 29 cells. In addition, two casein/phosvitin kinases have been identified in both cell lines with phosphorylating activity similar to the total histone kinases. In the nuclear extract two cyclic AMP-independent histone kinases have been found with electrophoretic mobility differing from the cytoplasmic enzymes. Also, two phosvitin/casein kinases specifically nuclear, due to their chromatographical and electrophoretical behavior, have been characterized. (Author abstract) (25 Refs)

24. PHOSPHORYLATION OF SYNTHETIC PEPTIDES BY A TYROSINE PROTEIN KINASE FROM THE PARTICULATE FRACTION OF A LYMPHOMA CELL LINE.

Casnellie JE, Harrison ML, Pike LJ, Hellstrom KE, Krebs EG
Howard Hughes Medical Inst. Lab., Dept. Pharmacology, Univ. Washington, Seattle, WA, 98195
Proc Natl Acad Sci USA; 79(2):282-286 1982

The particulate fraction from a lymphoma cell line, LSTRA, was found to contain an apparent high level of tyrosine protein kinase activity. When this fraction was incubated with [gamma-32P]ATP in the presence of 10 mM MnCl2, hydrolyzed, and assayed, 70-80% of the radioactivity recovered in phosphoamino acids was in phosphotyrosine. Gel electrophoresis of the proteins showed that a large portion of the 32P was in a single protein with a mol wt of approx 58,000. The phosphorylated residue in this protein was identified as phosphotyrosine. Detergent extracts of the particulate fraction from LSTRA cells contained both the Mr 58,000 protein and the enzyme responsible for its phosphorylation. These extracts were found to catalyze the phosphorylation of the tyrosine residue in the synthetic peptide, Ile-Glu-Asp-Asn-Glu-Tyr-Thr-Ala-Arg-Gln-Gly, corresponding to the sequence around the tyrosine that is phosphorylated in pp60src; the Km for the peptide in this reaction was 5 mM. High-performance liquid chromatography was used to assay for this phosphorylation. A second peptide was synthesized that contained two additional arginine residues whose presence permitted the phosphorylation of the peptide to be measured by a simple assay using phosphocellulose paper. The Km for this peptide was 3-4 mM, indicating that the presence of the additional arginine residues did not alter the apparent affinity of the kinase for the peptide. (Author abstract) (24 Refs)

25. CYTOSOLIC MALIC DEHYDROGENASE ACTIVITY IS ASSOCIATED WITH A PUTATIVE SUBSTRATE FOR THE TRANSFORMING GENE PRODUCT OF ROUS SARCOMA VIRUS.

Rubsamen H, Saltenberger K, Friis RR, Eigenbrodt E
Paul-Ehrlich-Institut, Paul-Ehrlich-Strasse 42-44, Frankfurt am Main, W. Germany
Proc Natl Acad Sci USA; 79(2):228-232 1982

A cellular protein of apparent Mr 34,000-36,000 was suggested as a possible physiological substrate for the protein kinase (EC 2.7.1.37) activity associated with the transforming gene product of Rous sarcoma virus. We find this protein to migrate with an apparent Mr of 38,000 in NaDodSO4/polyacrylamide gels. It was not separable from cytosolic malic dehydrogenase activity when purified by chromatography on DEAE-Sephacel, hydroxylapatite, poly(A)-Sepharose, and blue Sepharose, by gel filtration, and by isoelectric focusing. The Mr 38,000 protein as well as cytosolic malic dehydrogenase activity focused with a pI of 7.5. In gel filtration experiments, both displayed an apparent native Mr of 68,000. The malic dehydrogenase activity contained in homogeneous preparations of the Mr 38,000 protein had a specific activity of up to 130 units/mg of protein. The recovery of the enzyme was 5-10% of the activity in the extract. Antiserum against the Mr 38,000 protein inactivated the malic dehydrogenase activity associated with the Mr 38,000 protein. (Author abstract) (43 Refs)

26. BACTERIAL EXPRESSION OF AN ENZYMATICALLY ACTIVE PROTEIN ENCODED BY RSV SRC GENE.

McGrath JP, Levinson AD
Dept. Molecular Biology, Genentech, Incorporated, 460 Point San Bruno Blvd., South San Francisco, CA, 94080
Nature; 295(5848):423-425 1982

A bacterial plasmid was constructed which is capable of expressing in Escherichia coli a protein (p60) closely related in size and immunological properties to a 60,000-molecular wt (Mr) phosphoprotein (pp60src). The p60 protein appeared to be the translational product of the src gene under the control of the trp promoter. Total cellular proteins were analyzed in E coli transformed with recombinant vector pBR322, pHGH207-

1, or pSRCex16. No overall change in the protein profile occurred in cells transformed with pBR322. In cells transformed with pHGH207-1, a prominent protein of 22,000 Mr appeared. Only in pSRCex16-transformed cells, was a 60,000 Mr p60 protein induced; p60 appeared selectively during late growth phase. When induced, p60 represented approx 5% of total bacterial protein. Further characterization of the protein was performed. Protein kinase (PK) activity was assayed in recombinant E coli strain harboring either pBR322 or the p60 expression plasmid (pSRCex16). PK activity was present only in bacteria expressing p60; PK activity, when expressed, was tyrosine-specific. The finding of a tyrosine-specific PK activity in a highly purified bacterially-derived preparation of p60 lended support to the conclusion that this enzymatic activity represents an intrinsic property of the molecule. (35 Refs)

27. POLYOMA T ANTIGENS.

Eckhart W
Tumor Virology Lab., Salk Inst., San Diego, CA, 92138
Adv Cancer Res; 35:1-25 1981

Current knowledge on polyoma T proteins is reviewed, including the role of these viral-coded proteins in lytic infection of mouse cells, DNA nucleotide sequences (coding and regulatory signals), mutations affecting the T antigens, and cell transformation and tumorigenicity. The T antigens are compared structurally with SV40 in terms of nucleotide sequences. The roles of the large, small, and medium T antigens in lytic infection and transformation are discussed. The functions associated with small and medium T antigens, particularly attempts to detect protein kinase activity associated with polyoma T, are reviewed. (110 Refs)

28. EFFECT OF NONPROTEIN CHROMOPHORE (NPC) FROM NEOCARZINOSTATIN (NCS) ON CHROMATIN PROTEIN PHOSPHORYLATION (MEETING ABSTRACT).

Koyama K, Sato T, Kioke T, Ohtsuki K, Isida N

Department of Bacteriology, Tohoku University School of Medicine, Sendai, Japan

Proceedings of the Japanese Cancer Association, 40th Annual Meeting, October 1981, Sapporo, Japan. The Association 415 pp., 1981.

Low levels of NPC from NCS, which is an antitumor antibiotic, greatly inhibited in vitro protein phosphorylation by nuclear protein kinases. We found that phosphorylation of five distinct chromatin proteins in the 0.5 m NaCl extracts prepared from mouse spleen cell chromatin were insensitive to NPC. Two polypeptides were identified as Histone HI. (no Refs)

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AUTHOR INDEX WITH CITATION NUMBERS

Ait-Ahmed O 23
Almendral A 5
Anderson WB 19
Andreis PG 2
Armato U 2
Azzara A 1
Bakkeren JA 15
Bankhurst AD 18
Bill G 12
Bodwin JS 8
Brown EM 3
Buchanan RL 11
Burckhardt P 12
Casnellie JE 24
Chakmakjian ZH 4
Cho-Chung YS 8
Clarebrough JK 7
De Abreu RA 15
De Bruyn CH 15
Eckhart W 27
Eigenbrodt E 25
Elias L 18
Eppenberger U 5
Evain D 19
Fridkin M 13
Friis RR 25
Fukushima M 6
Gartemann A 17
Gottlieb P 13
Gumbiner B 9
Hamman HC 10
Handschin J 5
Harrison ML 24
Hellstrom KE 24
Hilz H 17
Hirayama PH 8
Huber P 5
Isida N 28
Jacquet AF 12
Kelly RB 9
Kinzel V 21
Kioke T 28
Koyama K 28
Krebs EG 24
Kubler D 21
Kung W 5
Ledford BE 10
Lehotay DC 16
Levey BA 16
Levey GS 16
Levinson AD 26
Lieberman ZH 4
Liu AY 20
Longmire J 18
Mangeat P 23
Mantero F 2
Marchis-Mouren 23
Marvaldi J 23
Matthews JL 4
McGrath JP 26
Notario A 1
Ohtsuki K 28
Ota K 6
Perversi L 1
Petrucci E 1
Pike LJ 24
Plet A 19
Pricca D 1
Pyerin W 21
Rogerson BJ 16
Rubsamen H 25
Saltenberger K 25
Sato T 28
Schretlen ED 15
Schwartz JP 14
Schwoch G 17
Snyder HW 22
Steward AM 7
Stone MJ 4
Tice G 11
Van Baal JM 15
Weber W 17
Wielckens K 17
Wilson A 7
Zocchi M 1

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In this issue:

- **REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES** 1-7
- **REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES** 8
- **CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES** 9-15
- **PROTEIN KINASE STUDIES** 16-27
- **OTHER RELATED STUDIES** 28-34

REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES

1. EFFECTS OF CYCLIC AMP ON GROWTH AND DIFFERENTIATION OF RAT RETINOBLASTOMA-LIKE TUMOR CELLS IN VITRO.

Nishida T, Mukai N, Solish SP, Pomeroy M
Dept. Ophthalmology, Osaka Univ. Medical Sch., 1-1-50 Fukushima, Fukushima-Ku, Osaka 553, Japan
Invest Ophthalmol Vis Sci; 22(2):145-156 1982

Effects of cyclic AMP analogs and phosphodiesterase of RAO 188 cells were investigated. The cell line was derived

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from a retinoblastoma-like rat tumor. 14C-Thymidine and 3H-uridine incorporation by RAO cells was inhibited by 1 milliM dibutyryl cyclic AMP, and inhibition reached a maximum at 5 milliM. Precursor incorporation was also inhibited by 8-bromocyclic AMP, theophylline, and aminop hylline, but not by dibutyryl cyclic guanosine monophosphate. Further observations indicated that dibutyryl cyclic AMP inhibited both DNA and RNA synthesis and that inhibition was irreversible after 10 days of incubation. Neurite-like processes developed shortly after cyclic AMP analog treatment and formed a network after 24 hr; ultrastructure was well organized and characterized by microvillous projections and intercellular junctional complexes. Results show that treatment which increases intracellular cyclic AMP concentration halts growth of RAO rat retinoblastoma-like tumor cells in culture and facilitates their morphologic and biochemical differentiation. (21 Refs)

2. CYCLIC NUCLEOTIDE MODULATION OF IN VITRO MORPHOLOGICAL TRANSFORMATION OF SYRIAN HAMSTER CELLS.

Mironescu SG, Epstein SM, DiPaolo JA
Dept. Pathology, Univ. South Carolina Sch. Medicine, Columbia, SC, 29208
Cancer Res; 42(4):1274-1278 1982

The cyclic nucleotides, cyclic adenosine 3':5'-monophosphate (cAMP) and cyclic guanosine 3':5'-monophosphate (cGMP) or their dibutyryl and monobrominated derivatives, may either increase or decrease morphological transformation of Syrian hamster embryo cells exposed to N-methyl-N'-nitro-N'-nitrosoguanidine (MNNG). The effect on transformation is primarily a function of the parent cyclic nucleotide and the duration of exposure to the nucleotides. At concentrations of 5 mM or larger for a minimum 24-hr exposure cAMP, cGMP, and their congeners reduced the colony-forming ability of nontransformed Syrian hamster embryo cells not exposed to MNNG; however, cGMP and its derivatives caused less toxicity than cAMP or its analogs. cAMP and its monobrominated and dibutyrylated derivatives decreased the transformation frequency associated with MNNG irrespective of whether the various adenylnucleotides were administered before or after MNNG. The greatest inhibitory effect on MNNG-induced transformation was obtained with N6,O2-dibutyryl cyclic adenosine 3':5'-monophosphate followed in order by 8-bromocyclic adenosine 3':5'-monophosphate and cAMP. At equimolar doses, the dibutyryl and brominated analogs of cGMP but not unsubstituted cGMP enhanced transformation when administered prior to exposure of the Syrian hamster embryo cells to MNNG but reduced the transformation frequency when added after MNNG. The enhancing and inhibitory effects of the guanine cyclic nucleotide-induced alteration of MNNG-associated transformation frequencies were dose and time dependent and occurred in the order N6,O2-dibutyryl cyclic guanosine 3':5'-monophosphate greater than 8-bromocyclic guanosine 3':5'-monophosphate very much greater than cGMP. Butyric acid neither diminished nor increased MNNG-induced transformation frequency. The latter suggests that butyrate ions formed by metabolism of the cyclic nucleotide analogs were not a factor in the observed alterations of trans formation frequency. (Author abstract) (35 Refs)

3. MODIFICATION OF RADIATION-INDUCED DIVISION DELAY BY CAFFEINE ANALOGUES AND DIBUTYRYL CYCLIC AMP.

Kimler BF, Snyder MH, Rowley R, Schneiderman MH
Lab. Experimental Radiation Oncology, Thomas Jefferson Univ. Hosp., Philadelphia, PA, 19107
Int J Radiat Biol; 41(1):47-58 1982

The mitotic selection procedure for cell cycle analysis was utilized to investigate the concentration-dependent modification of radiation-induced division delay in Chinese hamster ovary (CHO) cells by methyl xanthines (caffeine, theophylline, and theobromine) and by dibutyryl cyclic AMP. The methyl xanthines (concentrations from 0.5 to 1,000 ug/ml) all reduced radiation-induced division delay with the effect being linear between approximately 100 and 1,000 ug/ml. After doses of 100-300 rad, delay was reduced by 75, 94 or 83% at 1,000 ug/ml for each drug, respectively. However, the addition of dibutyryl cyclic AMP had an opposite effect: radiation-induced delay was increased by the concentration range of 0.3 to 300 ug/ml. These results indicate that in mammalian cells the control of cell cycle progression and the modification of radiation-induced division delay are not simply related to intracellular levels of cyclic AMP. Rather, there appear to be at least two competing mechanisms which are differentially affected by caffeine analogues or by direct addition of dibutyryl cyclic AMP. The direct effect of caffeine and the methyl xanthines on membrane calcium permeability is considered. (Author abstract) (31 Refs)

4. COMPARISON OF THE EFFECTS OF 3-ISOBUTYL-1-METHYLXANTHINE AND ADENOSINE CYCLIC 3':5'-MONOPHOSPHATE ON THE INDUCTION OF SKIN TUMORS BY THE INITIATION-PROMOTION PROTOCOL AND BY THE COMPLETE CARCINOGENESIS PROCESS.

Perchellet JP, Boutwell RK
McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI, 53706
Carcinogenesis; 3(1):53-60 1982

Topical application of either 5 umol of 3-isobutyl-1-methylxanthine (IBMX) or 0.25 umol of adenosine cyclic 3':5'-monophosphate (cyclic AMP) to the initiated skin of the mouse prior to each promotion with 8.5 nmol of 12-O-tetradecanoylphorbol-13-acetate (TPA) inhibited the formation of papillomas and carcinomas. Combined treatments including IBMX and cyclic AMP caused additive reduction of the incidence of skin papillomas and carcinomas promoted by TPA. A good correlation was observed between the reduction of the tumor incidence by IBMX and cyclic AMP and their inhibition of TPA-stimulated polyamine, RNA, protein, and DNA synthesis. Since repeated treatments with these agents before or after initiation with a single subcarcinogenic dose of 7,12-dimethylbenz[a]anthracene (DMBA) did not alter significantly the development of skin tumors, the present results suggest that, in the two-step initiation-promotion protocol, both IBMX and cyclic AMP treatments may decrease specifically the promoting stimulus of skin carcinogenesis. The same doses of IBMX and cyclic AMP inhibited the accumulation of polyamines and the increase in macromolecular synthesis observed in DMBA-treated skin, but their effect on DMBA-induced skin carcinogenesis was dependent upon the protocol used and the dose of carcinogen applied. IBMX and cyclic AMP treatments failed to inhibit the induction of skin tumors by weekly applications of 0.2 umol of DMBA. In contrast to the inhibitory effect of cyclic AMP treatment, IBMX enhanced the carcinogenic response to a single topical application of 3.6 umol of DMBA. The opposite effects of these agents on the carcinogenicity of DMBA correlated well with their different alteration of DMBA-induced unscheduled DNA synthesis in vitro. Cyclic AMP (0.5 mM) enhanced, whereas IBMX (0.5 mM) inhibited, the DMBA-induced incorporation of labeled precursor into DNA of isolated epidermal cells during incubation in the presence of hydroxyurea. Therefore, it is suggested that the different modulation of DMBA carcinogenesis by IBMX and cyclic AMP may result from concomitant effects on both the initiating and promoting components of the carcinogen. (Author abstract) (41 Refs)

5. HUMAN PANCREATIC ADENOCARCINOMA LINE CAPAN-1 IN TISSUE CULTURE AND THE NUDE MOUSE. MORPHOLOGIC, BIOLOGIC, AND BIOCHEMICAL CHARACTERISTICS.

Kyriazis AP, Kyriazis A, Scarpelli DG, Fogh J, Rao MS, Lepera R

Dept. Pathology, Univ. Cincinnati, 231 Bethesda Ave., Cincinnati, OH, 45267

Am J Pathol; 106(2):250-260 1982

Human pancreatic ductal adenocarcinoma line Capan-1 was studied in tissue culture and the nude mouse. In tissue culture, the neoplastic cells grew as large epithelial-like mucin-producing cells. Sc and ip transplantation of neoplastic cells into nude mice resulted in tumor formation characterized by marked invasiveness and distant metastases. Histologically, the tumor appeared as a well-differentiated mucin-producing adenocarcinoma morphologically resembling the tumor of origin. Chromosomal analysis showed a human karyotype with a chromosome number between 51-61. Lactate dehydrogenase and beta2-microglobulin used as tumor markers were present in both tissue culture and the serum of tumor-bearing mice. The neoplasm, which was characterized by an increased level of cAMP, had lost completely the ability to respond to secretin stimulation. The tumor grown in the nude mouse was resistant to treatment with 5-fluorouracil, behavior identical to that of the original tumor. Diphtheria toxin resulted in complete tumor destruction. Because Capan-1 tumor grown in the nude mouse shows morphologic, biologic, and biochemical characteristics similar to the tumor of origin, it may be an invaluable tool in furthering understanding of the biology of human pancreatic cancer. (Author abstract) (41 Refs)

6. RADIOPROTECTIVE PROPERTIES AND MECHANISM OF ACTION OF CYTOTOXIN FROM THE VENOM OF NAJA OXIANA EICHWALD.

Mukhamedzhanov KhS, Memetov FIu, Khalikov SK, Khudaikulov ZS

Inst. Biochemistry, Tashkent, USSR

Radiobiologiia; 21(6):918-922 1981

The radioprotective properties of cytotoxin isolated from the venom of Naja oxiana Eichwald were studied in random-bred mice, rats, and rabbits. The ip administration of cytotoxin (1 mg/kg) 1 hr prior to irradiation increased the survival and prolonged the lifespan of irradiated animals. The radioprotective effect of cytotoxin was associated with normalization of the cyclic AMP system. (13 Refs) (In RUS)

7. EFFECT OF PIRACETAM AND ISOPROTERENOL ON RATS WITH INDUCTION TUMORS.

von Metzler A, Nitsch C

Dept. Neurobiology, Max-Planck-Institut fur Hirnforschung, D-6000 Frankfurt/M., W. Germany

Naturwissenschaften; 69(1):48 1982

Tumor remission after administration of piracetam and isoproterenol (IP) in rats with 3-MC sarcoma (tumor diameter 0.3-3.0 cm) is reported. Cyclic AMP (cAMP) could play a part in transmitting information from tumor to CNS and the reverse, indicated by IP increase of the anticarcinogenic effect of a neuropharmacon. (11 Refs)

REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES

8. CHARACTERIZATION OF A NEW TYPE OF THYMOMA VARIANT SUPERSENSITIVE TO DEXAMETHASONE.

Mermod JJ, Intriere L, MacLeod CL, Bourgeois S

(c/o Bourgeois) Regulatory Biology Lab., Salk Inst., P.O. Box 85800, San Diego, CA, 92138

J Steroid Biochem; 15:25-34 1981

Some of the properties of dexamethasone supersensitive (Dexss) clones derived from two murine T-cell lines, W7 and T1M1, are described. In the presence of 10(-7) M dexamethasone (Dex), both supersensitive variants are killed faster than their parental line, but the phenotype appears stable. Both Dexss variants have the same number of glucocorticoid receptor sites/cell: approx 37,000 Dex binding sites/cell in W7 line and its Dexss variants, and 31,000 sites in the T1M1 line and its Dexss variant. The affinity of these receptors for dexamethasone appears also unchanged in the Dexss variants. No significant difference in the rate of phosphorylation between the parental W7 line and the Dexss variant, and no significant effect of Dex treatment were noticed. Results of lactate and glycogen production studies indicate that no change along the pathway accounts for the supersensitive phenotype of the W7 Dexss variant, and that the pathways are not affected by glucocorticoids in these cells. The supersensitive variant Dexss W7 is more sensitive to cyclic (c)AMP than the parental line; they are approx 10x more sensitive than both T1M1 parental line and the Dexss variant to cAMP. In the presence of 10(-7) M Dex, the addition of calcium has no significant effect on the cell density reached after 24 hr either by the W7 line or its Dexss variant or by T1M1 line and its Dexss variant. General changes in membrane permeability do not account for the supersensitive phenotype of the W7 Dexss variant. (29 Refs)

CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES

9. INHIBITION OF BETA-ADRENERGIC STIMULATION OF LYMPHOCYTE ADENYLATE CYCLASE BY PHORBOL MYRISTATE ACETATE IS MEDIATED BY ACTIVATED MACROPHAGES.

Novogrodsky A, Patya M, Rubin AL, Stenzel KH
Rogoff-Wellcome Medical Res. Inst., Beilinson Medical Center, Petah-Tikva, Israel
Biochem Biophys Res Commun; 104(2):389-393 1981

The in vitro effect of phorbol-12-myristate-3-acetate (PMA) on isoproterenol-stimulated adenylate cyclase in human peripheral blood mononuclear cells (PBM) was investigated with radioimmunoassay for cyclic AMP. PMA inhibited the isoproterenol-stimulated adenylate cyclase system in PBM but not in purified T-cells or in membrane preparations from turkey erythrocytes. Oxygen-derived radicals produced by PMA-activated macrophages evidently caused the inhibition. (12 Refs)

10. CHARACTERIZATION OF A NEWLY ESTABLISHED HUMAN GASTRIC CANCER CELL LINE HGT-1 BEARING HISTAMINE H2-RECEPTORS.

Laboisse CL, Augeron C, Couturier-Turpin MH, Gespach C, Cheret AM, Potet F
Laboratoire de Biologie et de Physiologie des Cellules Digestives, U 239 INSERM, Faculte de Medecine Xavier Bichat, 16 rue Henri Huchard, 75018 Paris, France
Cancer Res; 42(4):1541-1548 1982

A human gastric adenocarcinoma cell line, HGT-1, was established in vitro from the primary tumor of a 60-yr-old patient. Histological examination of the tumor revealed a poorly differentiated adenocarcinoma. Primary tumor cells were cloned in soft agarose and gave rise to tumor colonies. The procedures enabling us to form a continuous cell line from the agarose colonies are described. The cultured cells grew as monolayers of closely apposed polygonal cells with a population-doubling time of 19.48 -1.20 (SE) hr during exponential growth at passage 59. They had an epithelial morphology. Ultrastructural studies revealed the presence of microvilli and tight junctions. The HGT-1 cell line is tumorigenic in nude mice and has a hyperdiploid karyotype with a modal number of 57 chromosomes. It exhibits numerous marker chromosomes. These human gastric epithelial cells do not secrete mucus or carcinoembryonic antigen. They exhibit functional histamine H2-receptors mediating cellular cyclic adenosine 3':5'-monophosphate production and adenylate cyclase activation. In conclusion, the use of a soft-agarose clonogenic assay permitted us to develop a cancer cell line without the problems of fibroblastic cell contamination. The existence of histamine H2-receptors on gastric HGT-1 cells stresses the importance of this line as a model for studies of regulatory mechanisms involved in gastric secretion. (Author abstract) (39 Refs)

11. ADENYLATE CYCLASE SYSTEM IN CELLS OF EHRLICH ASCITES CARCINOMA SENSITIVE AND RESISTANT TO 5-FLUOROURACIL.

Shlemkevich MP, Iurkiv VA, Melikhov VI
Medical Inst., Lvov, USSR
Ukr Biokhim Zh; 54(1):76-79 1982

Activity of adenylate cyclase (AC) was studied in Ehrlich ascites carcinoma cells resistant and sensitive to 5-fluorouracil. Resistant cells had higher AC activity and lower phosphodiesterase activity than sensitive cells. The content of cyclic adenylate in resistant cells was significantly higher than that in the sensitive cells. Sodium fluoride increased AC activity both in resistant and sensitive cells. Prostaglandin E2 did not affect AC activity in sensitive cells and produced a 2x increase of AC activity in resistant cells. (11 Refs) (In RUS)

12. FUNCTION AND REGULATION OF METHIONINE(5)-ENKEPHALIN AND ITS RECEPTORS IN MURINE NEUROBLASTOMA CELLS.

Gilbert JA, Knodel EL, Stenstrom SD, Richelson E
(c/o Richelson), Mayo Clinic and Mayo Foundation, Rochester, MN, 55905
J Biol Chem; 257(3):1274-1281 1982

A homogeneous class of enkephalin receptors found in murine neuroblastoma clone N1E-115 has been confirmed using a centrifugation assay employing cellular membranes. In intact N1E-115 cells, synthetic methionine(5)-enkephalin inhibited prostaglandin E1-induced intracellular cyclic AMP formation in a naloxone-sensitive manner. Upon demonstrating intracellular methionine(5)-enkephalin immunocytochemically, analyses of crude N1E-115 extract were made by radioimmunoassay or opiate receptor binding assay following fractionation by molecular sieve chromatography and high pressure liquid chromatography on a mu-Bondapak C18 column. Extracted methionine(5)-enkephalin immunoreactive material behaved similarly to synthetic methionine(5)-enkephalin in these analyses. Growth curve studies of the N1E-115 cells indicated that the quantity of methionine(5)-enkephalin immunoreactive material synthesized per milligram of cellular protein and the max number of enkephalin receptor sites per milligram of membrane protein increased as the cells progressed from logarithmic to stationary phase, with no change in the apparent affinity of the enkephalin receptors for [3H]methionine(5)-enkephalin. These data suggest that adrenergic clone N1E-115 has functional methionine(5)-enkephalin membrane receptors, that this clone synthesizes methionine(5)-enkephalin, and that both the enkephalin receptor number and the content of stored methionine(5)-enkephalin are regulated with respect to cell division. (Author abstract) (40 Refs)

13. ANTITUMOR AGENTS XLVII: THE EFFECTS OF BISBRUSATOLYL MALONATE ON P-388 LYMPHOCYTIC LEUKEMIA CELL METABOLISM.

Hall IH, Liou YF, Lee KH, Okano M, Chaney SG
Div. Medicinal Chemistry, Sch. Pharmacy, Univ. North Carolina at Chapel Hill, Chapel Hill, NC, 27514
J Pharm Sci; 71(2):257-262 1982

Bisbrusatolyl malonate, which was shown previously to be active against P-388 lymphocytic leukemia cell growth, was investigated for inhibitory effects on nucleic acid and protein synthesis. DNA and RNA synthesis as well as protein synthesis were markedly inhibited at 10, 25 and 50 umole final concentrations in vitro. The major sites of inhibition of nucleic acid synthesis appeared to be DNA polymerase, messenger RNA polymerase and transfer RNA polymerase, orotidine-5'-monophosphate decarboxylase, phosphoribosyl pyrophosphate amino transferase, and dihydrofolate reductase. Moderate inhibition of nucleotide kinase activities and oxidative phosphorylation processes occurred after drug treatment. Cyclic adenosine monophosphate levels were reduced. Protein synthesis was inhibited during the elongation step of peptide synthesis. The data suggested that bisbrusatolyl malonate interfered with the peptide bond formation. However, the ongoing polypeptide synthesis must be completed before the drug can bind to the ribosome effectively. (Author abstract) (41 Refs)

14. CHOLERA TOXIN COVALENTLY LINKED WITH HORSERADISH PEROXIDASE DOES NOT ACTIVATE THE ADENYLATE CYCLASE OF NEUROBLASTOMA CELLS.

Charalampous FC, Gonatas JO, Van Heyningen S, Gonatas NK
Dept. Biochemistry and Biophysics, Univ. Pennsylvania Sch. Medicine, Philadelphia, PA, 19104
Exp Cell Res; 137(2):449-452 1982

Cholera toxin covalently linked with horseradish peroxidase (HRP) looses its activating effect on the adenylate cyclase of cultured murine neuroblastoma cells. (Author abstract) (17 Refs)

15. VITAMIN D NUTRITION INCREASES SKIN TYROSINASE RESPONSE TO EXPOSURE TO ULTRAVIOLET RADIATION.

Pavlovitch JH, Rizk M, Balsan S
Hopital des Enfants-Malades, Tour Technique 6eme etage, 149 rue de Sevres, 75015 Paris, France
Mol Cell Endocrinol; 25(3):295-302 1982

UV-induced melanogenesis (as assessed by skin tyrosinase activity) was greater in vitamin-D-fed rats than in vitamin-D-deficient rats. The increase in skin tyrosinase activity in response to UV irradiation was preceded by an increase in skin cyclic AMP levels, and this increase was greater in vitamin-D-treated animals than in vitamin-D-deficient rats. The effect of vitamin D on skin tyrosinase activity was potentiated by the phosphodiesterase inhibitor 3-isobutyl-methyl-xanthine. (24 Refs)

PROTEIN KINASE STUDIES

16. TRANSFORMED CELLS EXHIBIT ALTERED RESPONSE TO DB CYCLIC AMP-MEDIATED MODULATION OF PROTEIN PHOSPHORYLATION AND DIFFERENT ENDOGENOUS PHOSPHOPROTEIN ACCEPTORS.

Rieber MS, Rieber M
(c/o Rieber) Center Microbiology and Cell Biology, Inst. Venezolano de Investigaciones Centrificas, Apartado 1827, Caracas 1010-A, Venezuela
Cancer Biochem Biophys; 5(3):163-167 1981

Detergent solubilized particulate fractions (DSF) and cytoplasmic fractions (CF) derived from NRK cells transformed with the temperature-sensitive ts24 derivative of Rous sarcoma virus and grown at 33 C (malignant phenotype) and 39 C (normal phenotype) were compared. Protein kinase activities were in the following order: DSF, transformed greater than or equal to DSF, phenotype normal greater than or equal to CF, transformed greater than or equal to CF, phenotype normal. Incubation of CF of morphologically normal cells grown for 48 hr at 39 C with ATP-gamma-32P led to phosphorylation mostly in components of 60,000 and 68,000 daltons; in vitro exposure of extracts to dibutyryl cyclic AMP increased labeling of the 60,000 dalton component and decreased labeling in the 68,000 dalton component. A similar experiment with CF of phenotypically transformed cells grown at 33 C yielded 140,000 and 38,000 dalton components, both of which showed decreased labeling when the experiment was repeated with cyclic AMP preincubation. DSF of phenotypically normal cells labeled preferentially in 38,000, 65,000, and 94,000 dalton regions, and these preferences did not change with cyclic AMP. DSF of phenotypically transformed cells labeled mainly in the 65,000 dalton region; with cyclic AMP preincubation the 65,000 dalton labeling decreased and labeling increased in the 220,000 dalton region. Failure of transformed cells to phosphorylate some substrates may be due to a transformation-induced alteration in the availability of endogenous protein acceptors for the phosphorylation process, and not to a low level of protein kinase activity. (13 Refs)

17. ANALYSIS OF THE SEQUENCE OF AMINO ACIDS SURROUNDING SITES OF TYROSINE PHOSPHORYLATION.

Patschinsky T, Hunter T, Esch FS, Cooper JA, Sefton BM
Tumor Virology Lab., Salk Inst., P. O. Box 85800, San Diego, CA, 92138
Proc Natl Acad Sci USA; 79(4,part1):973-977 1982

We have identified the single phosphorylated tyrosine in p60src, the transforming protein of Rous sarcoma virus, as part of the sequence NH2-Arg-Leu-Ile-Glu-Asp-Asn-Glu-Tyr(P)-Thr-Ala-Arg COOH. Therefore, this is a sequence that is recognized efficiently by a tyrosine protein kinase in vivo. Phosphorylation of tyrosine in cellular proteins appears to play a role in malignant transformation by four classes of genetically distinct RNA tumor viruses. Phosphorylated tyrosines in several other proteins resemble the tyrosine in p60src in that they are located 7 residues to the COOH-terminal side of a basic amino acid and either 4 residues to the COOH-terminal side of, or in close proximity to, a glutamic acid residue. Therefore, it is possible that these features play a role in the selection of sites of phosphorylation by some tyrosine protein kinases. However, several clear exceptions to this rule exist. (Author abstract) (38 Refs)

18. PROTEIN KINASE (PP67-INF) IN PLASMA AND TISSUE OF MICE WITH HIGH LEVELS OF CIRCULATING INTERFERON.

Hovanessian AG, Riviere Y, Robert N, Svab J, Chamaret S, Guillon JC, Montagnier L
Departement de Virologie, Institut Pasteur, 75724 Paris Cedex 15, France
Ann Virol; 132(2):175-188 1981

Mouse L-929 cells were treated with interferon (IFN) and the enhanced protein kinase activity manifested by the phosphorylation of the endogenous 67,000 mol wt phosphoprotein (pp67-IFN) was studied. Levels of circulating IFN, of pppA(2'p5'A)n synthetase (2-5A synthetase), of pp67-IFN kinase in the spleen and plasma of mice inoculated with Newcastle disease virus, of encephalomyocarditis virus, of poly(I)poly(C), or of poly(A)poly(U), were measured. Levels of these enzymes were enhanced in accordance with the level of circulating IFN, reaching the max 15-18 hr after IFN reached the peak for 2-5A synthetase, and 24 hr for pp67-IFN kinase. 2-5A synthetase could not b detected in the mice serum or plasma prepared with sodium citrate, even when they showed high levels of 2-5A synthetase and pp67-IFN kinase in their liver and spleen. Amino acids involved in the phosphorylation of pp67-IFN from three different sources were investigated. The pp67-IFN were phosphorylated mostly by their serine residues. Threonine residues were also phosphorylated but at much lower levels, and no phosphorylation of tyrosine residues was observed. The phosphorylations were carried out in vitro. Results indicate the significance of the pp67-IFN kinase measurement for testing the response of an organism to IFN or IFN-inducers, and the possible existence of a saturation point for the IFN action beyond which the tested system does not respond. (17 Refs)

19. CELLULAR SUBSTRATE FOR THE SRC GENE PRODUCT.

Kobayashi N
Dept. Microbiological Medicinal Chemistry, Tokyo Univ. Sch. Pharmacology, Tokyo, Japan
Tanpakushitsu Kakusan Koso; 26(10):1537-1553 1981

Current ideas concerning the phosphoprotein pp36K (mol wt 36,000), which is found in Rous sarcoma virus-infected cells, were presented. Its correspondence to viral carcinogenesis was discussed, and the following aspects were included bird sarcoma virus carcinogenic gene products, determination of src gene, src gene products, phosphorylation of p60src and its protein kinase activation, phosphorylation of thyroxin, and protein kinase activity carried by carcinogenic gene products other than src, intracellular marker proteins of src gene products, determination of pp36K, p36K (a non-phosphorylated precursor of pp36K), pp36K existing in cells other than chicken cells, in vitro phosphorylation of p36K, cellular elution using radio immune precipitation assay, unique damage in p36K phosphorylation reaction due to tumor -bearing rat serum, phosphorylated amino acids of pp36K, intracellular mechanism of pp36K, and intracellular marker proteins of pp60src other than p36K. (118 Refs) (In JPN)

20. CHARACTERIZATION OF IMMUNE TYPE INTERFERON (IFNGAMMA)-INDUCED CYTOPLASMIC PROTEIN KINASE ACTIVITY IN MOUSE L CELLS.

Ohtsuki K, Torres BA, Johnson HM
Dept. Microbiology, Univ. Texas Medical Branch, Galveston, TX, 77550
Biochem Biophys Res Commun; 104(2):422-429 1981

Mouse immune interferon (IFN-gamma) induced double-stranded RNA-dependent protein kinase activity in the cytoplasmic fraction of mouse L cells, measured by histone phosphorylation with (gamma-32P)ATP. The protein kinase activity was separated by column chromatography into three fractions of 100,000, 70,000, and 70,000 dalton mol wt. The fractions differed in Mg and Ca requirements, cyclic AMP activation, N-ethylmaleimide inhibition effect, and substrate requirements. (15 Refs)

21. MODULATION OF PROTEIN PHOSPHORYLATION IN HUMAN COLON ADENOCARCINOMA CELL MEMBRANE PREPARATIONS BY EPIDERMAL GROWTH FACTOR IN VITRO.

Zenisek SC, Fernandez-Pol JA
(c/o Fernandez-Pol) Nuclear Medicine Lab., Veterans Admin Medical Center and Dept. Medicine, Saint Louis Univ., St. Louis, MO, 63125
Int J Cancer; 29(3):277-282 1982

Incubation of membranes prepared from the human colon adenocarcinoma cell line LoVo in vitro with [gamma-32P]ATP demonstrated numerous components whose phosphorylation was stimulated several fold by epidermal growth factor (EGF). One major component of Mn 170 K, which was either undetectable or very weakly phosphorylated in the absence of EGF, was primarily affected by exposure to EGF. The phosphorylation of the 170 K Mr membrane component required the presence of Mn; Mg was ineffective. Although the phosphorylation of many LoVo membrane components was significantly modified by cAMP or dibutyryl-cAMP, none of these cyclic nucleotides appeared to be involved in the phosphorylation of the 170 K membrane component in the presence or absence of EGF. The phosphorylation system of the LoVo membranes efficiently utilized [gamma-32P]GTP in both the basal and EGF-stimulated reactions. All the membrane components phosphorylated in the absence or presence of EGF, except a band comigrating with the tracking dye front, were digested by trypsin. The possible glycoprotein nature of the 170 K dalton phosphoprotein was indicated by the fact that the 170 K dalton band comigrated with a periodic acid-Schiff base-stained band. These findings suggest that one of the biochemical steps in the mechanism of action of EGF in LoVo cells is enhanced phosphorylation of several membrane proteins, especially that of a glycoprotein of Mr 170 K, by a membrane-bound cyclic nucleotide independent protein kinase. (Author abstract) (23 Refs)

22. ALTERATION OF EPIDERMAL GROWTH FACTOR-DEPENDENT PHOSPHORYLATION DURING RAT LIVER REGENERATION.

Rubin RA, O'Keefe EJ, Earp HS

Cancer Cell Biology Pogram, Cancer Res. Center, Univ. North Carolina, Chapel Hill, NC, 27514

Proc Natl Acad Sci USA; 79(3):776-780 1982

Epidermal growth factor (EGF) stimulates membrane protein phosphorylation in a human cell line, A-431. The known hepatic mitogenic action of EGF and the reduction in EGF receptor number that occurs during liver regeneration led us to study whether EGF-dependent protein kinase activity was present in rat liver and whether its activity was altered after partial hepatectomy. Liver membranes preincubated with or without EGF, were phosphorylated (0 C, 15 sec) and subjected to NaDodSO4/polyacrylamide gel electrophoresis and autoradiography. In microsomal fractions, EGF at 5-2,000 ng/ml produced a dose-related stimulation of 32P incorporation into a single 170,000-dalton protein (p170). In plasma membranes, a similar EGF-dependent phosphorylation was present and was substantially enriched relative to the microsomal fraction. Acid hydrolysis of labeled microsomal fraction followed by phosphoamino acid determination revealed that EGF stimulated 32P incorporation into phosphotyrosine residues. The EGF-dependent phosphorylation of p170 was compared in microsomal fractions isolated from rats 36 hr after partial hepatectomy or sham operation. In the absence of EGF, in vitro labeling of p170 was similar. EGF stimulated the labeling of p170 in both groups, but the response was clearly diminished after partial hepatectomy. In the presence of EGF, the labeling of p170 in microsomal fraction from regenerating livers was only 47 -6% of that observed in membranes from sham-operated rats (p less than 0.005). Reduction of EGF-dependent phosphorylation during liver regeneration paralleled the loss of binding of 125I-labeled EGf. An increase in the EGF-dependent phosphorylation of a 130,000-dalton protein was also observed after partial hepatectomy. The increase in the amount of this phosphoprotein was roughly equal to the loss of EGF-stimulated p170 phosphorylation. Several additional proteins showed increased phosphorylation in membranes from partially hepatectomized rats. These findings indicate that alterations in membrane tyrosine residue phosphorylation occur during regulated growth in vivo. (Author abstract) (28 Refs)

23. ASSAYS OF CELL SURFACE PROTEIN KINASE: IMPORTANCE OF SELECTING CYTOPHILIC SUBSTRATES.

Kubler D, Pyerin W, Kinzel V
Abt. fur Molekulaire Pathologie, Institut fur Experimentelle Pathologie, Deutsches Krebsforschungszentrum, Im Neuenheimer Fedl 280, D-6900 Heidelberg 1, W. Germany
Eur J Cell Biol; 26(2):306-309 1982

The ability of phosvitin and histone to serve as substrates for possible protein kinase(s) at the cell surface was examined with regard to their suitability as indicators of such activity. While phosvitin did not interfere with the membrane barrier of HeLa cells, 3T3 and SV 3T3 cells, histone caused severe damage as indicated by both uptake of viability stains Trypan Blue and diamidino-phenylindol and by release of intracellular compounds such as lactate dehydrogenase, cAMP-dependent protein kinase(s), and metabolically prelabeled proteins. Where intactness of the cell membrane is prerequisite for verification of ecto-protein kinase, histone cannot be used as substrate. In contrast, we found that phosvitin is suitable for assays of cell surface located protein kinase activity. (Author abstract) (22 Refs)

24. MUTATION CAUSING PREMATURE TERMINATION OF THE POLYOMA VIRUS MEDIUM T ANTIGEN BLOCKS CELL TRANSFORMATION.

Templeton D, Eckhart W
Tumor Virology Lab., Salk Inst., San Diego, CA, 92138
J Virol; 41(3):1014-1024 1982

We used site-specific mutagenesis to introduce a termination codon, TGA, into the reading frame for the polyoma virus medium T antigen. We induced this mutation in a region of the polyoma genome in which the overlapping coding regions for the large and medium T antigens are translated in different reading frames. Therefore, the mutation terminated translation of the medium T antigen, but it caused only a single amino acid substitution in the large T antigen and did not affect the small T antigen. Cells infected by the mutant virus produced normal-size small and large T antigens. The infected cells produced a 28,000-dalton fragment of the 48,000-dalton medium T antigen, whose size and tryptic peptide map were consistent with its being a truncated N-terminal fragment terminating at the new termination codon of the mutant. Immunoprecipitates of mutant-infected cell extracts did not show medium-T-antigen-associated protein kinase activity. The mutant virus replicated normally in mouse 3T6 cells and induced cellular DNA synthesis in resting mouse 3T3 cells, but it failed to transform rat or hamster cells, as judged by focus formation and growth in agar. The mutant complemented a tsA mutant which affects the large T antigen for transformation, implying that the mutant defect for transformation was in the medium T antigen. These results imply that the small T antigen and the large T antigen together are insufficient to cause transformation and support the conclusion that the medium T antigen is essential for cell transformation by polyoma virus. (Author abstract) (31 Refs)

25. REGULATION OF PROTEIN KINASES ACTIVITY BY QUERCETIN IN EHRLICH ASCITES TUMOR CELLS.

Graziani Y, Chayoth R, Karny N, Feldman B, Levy J
Dept. Pathology, Univ. Colorado, Sch. Medicine, Denver, CO, 80262
Biochim Biophys Acta; 714(3):415-421 1982

Cyclic AMP-independent protein kinase activities from Ehrlich ascites tumor cell s, partially purified by DEAE-cellulose and phosphocellulose chromatography were inhibited by quercetin. The cyclic AMP in the tumor ascites cells and the cyclic AMP-dependent protein kinase activity from this tumor and from bovine and mouse tissues were unaffected by this drug. Since we reported that quercetin elevates cyclic AMP level in Ehrlich ascites tumor cells, this bioflavonoid may have a dual effect on the protein kinase activities in these cells, thus, increasing the cyclic AMP-dependent and decreasing the cyclic AMP-independent protein kinase activities. (Author abstract) (26 Refs)

26. CYTOCHEMICAL MARKERS OF BLADDER CARCINOGENESIS.

Wilson PD, Summerhayes IC, Hodges GM, Trejdosiewicz LK, Nathrath WJ
Physiologisches Institut der Universitat Munchen, Pettenkoferstrasse 12, 8 Munchen 2, W. Germany
Histochem J; 13(6):989-1007 1981

Enzyme cytochemical and immunocytochemical techniques were used at the light and electron microscope levels to study the distribution of potential enzyme and antigen markers of chemical transformation in rat and mouse bladders. In in vivo methylnitrosourea-induced rat tumors, alkaline phosphatase (AP) localization was normal on the external surface of the plasma membranes of some cells whereas in others AP was detected only on intracellular membranes. 5'-Nucleotidase (5NU) localization was abnormal in all cells, being seen on endoplasmic reticulum and nuclear membranes only, while in normal bladders only ectoenzyme localization was seen. After tumors were cultured as explants for 15 days, heterogeneity of AP and 5NU localization was seen on the plasma membranes. Enzyme production within some cells was variable while in other cells, only parts of the membrane reacted heavily. A bimodal pattern of AP localization was seen in transformed cell cultures and tumors of mouse bladder derived by in vitro treatment of explants with dimethylbenz(a)anthracene. Cells had either normal ectoenzyme reaction product or abnormal intracellular membrane reaction product. 5NU and adenosine diphosphatase were lost and an Mg.ATPase and a cyclic AMP-independent, calcium-insensitive 'protein phosphatase' were induced in transformed cell cultures. Immunocytochemical study revealed an epithelial antigen in the cytoplasm of both normal and transformed cells which was associated with reticular cytoplasmic ground substance, plasma membrane vesicles and cytoskeletal elements. (29 Refs)

27. CAUTIONS ON THE USE OF THE HEAT STABLE INHIBITOR OF PROTEIN KINASE: STUDIES WITH S49 LYMPHOMA CELLS.

Kanter JR, Brunton LL
Div. Pharmacology, Div. Cardiology M-013H, Sch. Medicine Univ., California San Diego, La Jolla, CA, 92093
J Cyclic Nucleotide Res; 7(4):259-268 1981

While the heat stable inhibitor of protein kinase (PKI, Mr = 11,000 daltons) specifically inhibits the activity of the free catalytic subunit of cyclic AMP-dependent protein kinase (cAMP-PK), the crude preparations of PKI which are often employed may produce unusual and anomalous effects. Explanations of these effects, a simple way to avoid them, and information on the variety of protein kinases in S49 lymphoma cells are presented. Crude PKI did not completely inhibit cAMP-PK in homogenates of S49 lymphoma cells, indicating that the observed increase in phosphorylation involved a kinase other than cAMP-PK. Purification of the crude preparations of PKI over diethylaminoethyl (DEAE) cellulose resulted in the removal of putative substrate proteins implied kinetically in the experiments with S49 lymphoma cell homogenates and detected by autoradiography of gels of reaction mixtures. Use of crude preparations of PKI is not advised; only material purified over DEAE cellulose should be used as a specific inhibitor of cAMP-dependent protein kinase activity. (18 Refs)

OTHER RELATED STUDIES

28. INTERFERON-INDUCED ANTIVIRAL ACTIONS IN FRIEND LEUKEMIA CELLS: ROLE OF MEMBRANE GANGLIOSIDES.

Belardelli F, Aliberti A, Santurbano B, Antonelli G, D'Agnolo G, Rossi GB
Servizio Biologico, Istituto Superiore di Sanita, Rome, Italy
Virology; 117(2):391-400 1982

The interactions between interferon (IFN) and membrane gangliosides have been analyzed in Friend leukemia cells (FLC). The GM1-cholera toxin (CT) model has been selected as a reference system for a high-affinity ganglioside-ligand binding. Two gangliosides have been detected in FLC extracts: a disialoganglioside (approx 95%) and a monosialoganglioside (approx 5%) which migrate respectively as GD1a and GM1. Unilammellar liposomes containing either mixed commercial gangliosides or GD1a or GM1 bind three to eight fold more IFN (both partially purified and pure) than gangliosides-free liposomes. Only 3-4% of the input IFN was bound, whereas GM1-containing liposomes bound almost 50% of [3H]acetyl-CT. Neuraminidase treatment of FLC, which converts GD1a-like into the GM1-like ganglioside, does not significantly modify the IFN-induced antiviral effects. CT binding to FLC as well as CT-induced elevation of endocellular cyclic AMP levels in FLC were instead markedly increased by the same neuraminidase treatment. Further, pretreatment of FLC with either GD1a or GM1 or gangliosides extracted from FLC did not change the IFN-induced establishment of the antiviral state. These data provide convincing evidence that FLC gangliosides do not represent the IFN high-affinity receptors nor specifically mediate IFN effects, even though they bind IFN to some extent. (Author abstract) (26 Refs)

29. MODULATION OF NATURAL KILLER SENSITIVITY OF MURINE TROPHOBLAST CELLS BY TUMOR PROMOTER AND INTERFERON.

Tanaka K, Chang KS
Lab. Cell Biology, NCI, Bethesda, MD, 20205
Int J Cancer; 29(3):315-321 1982

The in vitro established lines of murine placental trophoblast cells showed little membrane expression of H-2 antigens and a high degree of sensitivity to natural killer (NK)-cell-mediated cytotoxicity. This NK-sensitivity was decreased either by short-term treatment of these cells with murine interferon (IFN) or by culture for more than 4 days in the presence of a tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA). This effect of TPA was reversible after removal of TPA from the culture medium. However, IFN treatment of trophoblast cells cultured previously in TPA medium resulted in restoration of NK-sensitivity to the original level exhibited by cells untreated with IFN or TPA. The mechanism for this antagonistic effect is not clear. The decrease in NK-sensitivity of IFN- or TPA-treated trophoblast cells was not due to the loss of NK-recognition membrane structures, or to induction of prostaglandin which elevates cyclic-AMP levels. Inhibition of protein synthesis by cycloheximide restored the NK-sensitivity of TPA-treated trophoblast cells, indicating the possible contribution of de novo synthesized protein(s) which could confer NK-resistance. The TPA-treated trophoblast cells became more tumorigenic, producing larger tumors and a higher mortality than the untreated controls when inoculated into mice. The possible roles of NK cells in trophoblastic diseases and feto-maternal relationships are discussed. (Author abstract) (27 Refs)

30. ABNORMAL BONE AND PARATHYROID HISTOLOGY IN CARCINOMA PATIENTS WITH PSEUDOHYPERPARATHYROIDISM.

Sharp CF, Rude RK, Terry R, Singer FR
Dept. Medicine, LAC/Univ. Southern California Medical Center, Room 6442, 1200 North State St., Los Angeles, CA, 90033
Cancer; 49(7):1449-1455 1982

Postmortem bone and parathyroid gland histology in nine hypercalcemic cancer patients without bone metastases was compared to bone and parathyroid histology in 10 normocalcemic patients. Parameters of parathyroid function, including serum immunoreactive parathyroid hormone, acid base status, serum phosphate, and nephrogenous cyclic AMP were measured in the hypercalcemic group and compared to normals and to patients with primary hyperparathyroidism. Bone histology in all nine hypercalcemic cancer patients showed increased osteoclastic bone resorption and increased fibrous connective tissue in the bone marrow. Parathyroid glands were of normal size in all nine patients but contained little or no fat, one criterion of parathyroid hyperplasia. In the normocalcemic cancer patients only 2/10 had minimally increased bone resorption while 7/10 had decreased or absent stromal fat in the parathyroid glands. Despite the hyperplastic appearance of the parathyroid glands, serum biochemical parameters in the hypercalcemic cancer patients indicate a state of suppressed parathyroid function suggesting that the osteoclastic bone resorption is related to a humoral substance elaborated by the tumors which is distinct from parathyroid hormone. (Author abstract) (30 Refs)

31. CHARACTERIZATION OF AN EARLY INHIBITORY EFFECT OF GLUCOCORTICOIDS ON STIMULATED ADRENOCORTICOTROPIN AND ENDORPHIN RELEASE FROM A CLONAL STRAIN OF MOUSE PITUITARY CELLS.

Phillips M, Tashjian AH
(c/o Tashijian) Lab. Toxicology, Harvard Sch. Public Health, 665 Huntington Ave., Boston, MA, 02115
Endocrinology; 110(3):892-900 1982

The ability of glucocorticoid steroid hormones to inhibit release of ACTH and endorphin has been studied in the mouse pituitary tumor cell strain, AtT-20/D16v. Inhibition of release of both peptides was first detectable at 30 min (to 80-90% of control release) and was maximal (30-50% of control) by 3-4 hr of pretreatment with dexamethasone (1 x 10(-6) M). Glucocorticoids did not consistently affect basal hormone release, but they inhibited release stimulated four- to eightfold by hypothalamic extract, phorbol esters, or 8-Br-cAMP but not high K-stimulated release. The inhibitory effect was glucocorticoid specific and dose dependent with ED50S for dexamethasone, corticosterone, and cortisol close to their reported Kds for binding to the cytoplasmic glucocorticoid receptor. Inhibition of release was blocked by cycloheximide (1 x 10(-5) M), suggesting that protein synthesis was required. Experiments were performed to determine the site of action of glucocorticoids.

Steroid pretreatment for up to 6 hr had no effect on total intracellular content of ACTH or beta-endorphin, hormone biosynthesis, or intracellular and extracellular hormone degradation. These results show that the inhibitory action of steroids was not due to decreased hormone synthesis, as has been reported after longer periods (24-72 hr) of glucocorticoid treatment. Experiments with the tumor-promoting phorbol esters (used as model secretagogues) showed the pretreamtent of D16 cells with dexamethasone (1 x 10(-6) M) did not alter the binding of [3H]phorbol dibutyrate to its specific receptor on these cells, indicating that glucocorticoids do not act by interfering with binding of secretagogues to the cells. Furthermore, glucocorticoids did not affect the ED50 for stimulation of ACTH release by phorbol esters, but they reduced the magnitude of maximum response. We conclude that an early action of glucocorticoids on D16 cells is to inhibit the release of ACTH and beta-endorphin by a mechanism that is independent of the synthesis and degradation of the secretory peptides but which involves the synthesis of new protein. (Author abstract) (31 Refs)

32. ISOLATION OF PRIMARY CULTURES FROM POST-MORTEM SAMPLES OF HUMAN BRAIN.

West GM, Vaughan PF, Freshney RI, McNamee HB, Graham DI
Dept. Biochemistry, Univ. Glasgow, Glasgow G12 8QQ, Scotland
Biochem Soc Trans; 10(1):53-54 1982

Various marker enzymes (cyclic nucleotide phosphohydrolase, glycerol phosphate dehydrogenase, and glutamine synthetase) were assayed in rat C6 glioma cells and compared with primary cultures derived from human brain. Four cell lines studied (G-ATA, G-CCM, G-RTA and IJK) were grown from human gliomas; two (G-CCM and IJK) are glial fibrillary acidic protein-positive. Seven samples of fetal brain were also studied along with cultures from normal adult brain (which are glial fibrillary acidic protein-negative). (3 Refs)

33. THE 24-H URINARY CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE/CREATININE RATIO: AN USEFUL APPROACH TO THE DIAGNOSIS OF PARATHYROID DISORDERS AND FUNCTION.

Caniggia A, Nuti R, Galli M
Clinica Medica Generale, Universita di Siena, Piazza della Selva 7, 53100 Siena, Italy
J Endocrinol Invest; 4(3):281-287 1981

Determinations of urinary excretion of cyclic AMP are reported for patients with hypoparathyroidism, primary hyperparathyroidism before and immediately after surgery, normocalcemic hypercalcemia with recurrent nephrolithiasis, osteomalacia before and upon treatment with vitamin D or its metabolites, Paget's disease, and postmenopausal osteoporosis before and upon treatment with hypocalcemic drugs. Urinary calcium, phosphate, hydroxyproline and creatinine were also determined. (34 Refs)

34. THE BINDING OF [3H]ADENOSINE TO CELLS OF THE 108CC 15 NEUROBLASTOMA X GLIOMA HYBRID LINE.

Snell PH, Moses MA, Snell CR
MRC Neuroendocrinology Unit, Newcastle General Hosp., Westgate Road, Newcastle upon Tyne NE4 6BE, England
Biochem Soc Trans; 10(1):55-56 1982

Receptors for (3H)adenosine were demonstrated by high affinity binding on 108CC 15 hybrid neuroblastoma cells. Two receptors, one of high affinity and one of low affinity, were found. The density of both receptors varied with the growth of cells in culture, but were reasonably constant in cells in exponential growth and at 60-80% cover. Various analogues tested (cyclic AMP, ATP, NADP and 2',5'-adenyladenosine) did not displace the adenosine at the low affinity site. (11 Refs)

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AUTHOR INDEX WITH CITATION NUMBERS

Aliberti A 28
Antonelli G 28
Augeron C 10
Balsan S 15
Belardelli F 28
Bourgeois S 8
Boutwell RK 4
Brunton LL 27
Caniggia A 33
Chamaret S 18
Chaney SG 13
Chang KS 29
Charalampous FC 14
Chayoth R 25
Cheret AM 10
Cooper JA 17
Couturier-Turpi 10
D'Agnolo G 28
DiPaolo JA 2
Earp HS 22
Eckhart W 24
Epstein SM 2
Esch FS 17
Feldman B 25
Fernandez-Pol J 21
Fogh J 5
Freshney RI 32
Galli M 33
Gespach C 10
Gilbert JA 12
Gonatas JO 14
Gonatas NK 14
Graham DI 32
Graziani Y 25
Guillon JC 18
Hall IH 13
Hodges GM 26
Hovanessian AG 18
Hunter T 17
Intriere L 8
Iurkiv VA 11
Johnson HM 20
Kanter JR 27
Karny N 25
Khalikov SK 6
Khudaikulov ZS 6
Kimler BF 3
Kinzel V 23
Knodel EL 12
Kobayashi N 19
Kubler D 23
Kyriazis A 5
Kyriazis AP 5
Laboisse CL 10
Lee KH 13
Lepera R 5
Levy J 25
Liou YF 13
MacLeod CL 8
McNamee HB 32
Melikhov VI 11
Memetov FIu 6
Mermod JJ 8
Mironescu SG 2
Montagnier L 18
Moses MA 34
Mukai N 1
Mukhamedzhanov 6
Nathrath WJ 26
Nishida T 1
Nitsch C 7
Novogrodsky A 9
Nuti R 33
O'Keefe EJ 22
Ohtsuki K 20
Okano M 13
Patschinsky T 17
Patya M 9
Pavlovitch JH 15
Perchellet JP 4
Phillips M 31
Pomeroy M 1
Potet F 10
Pyerin W 23
Rao MS 5
Richelson E 12
Rieber M 16
Rieber MS 16
Riviere Y 18
Rizk M 15
Robert N 18
Rossi GB 28
Rowley R 3
Rubin AL 9
Rubin RA 22
Rude RK 30
Santurbano B 28
Scarpelli DG 5
Schneiderman MH 3
Sefton BM 17
Sharp CF 30
Shlemkevich MP 11
Singer FR 30
Snell CR 34
Snell PH 34
Snyder MH 3
Solish SP 1
Stenstrom SD 12
Stenzel KH 9
Summerhayes IC 26
Svab J 18
Tanaka K 29
Tashjian AH 31
Templeton D 24
Terry R 30
Torres BA 20
Trejdosiewicz L 26
Van Heyningen S 14
Vaughan PF 32
Von Metzler A 7
West GM 32
Wilson PD 26
Zenisek SC 21

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Cyclic Nucleotides

SERIES CB14 No. 82/08

AUGUST, 1982

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In this issue:

- **REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES** 1-8
- **REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES** 9-21
- **CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES** 22-29
- **CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS** 30
- **CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES** 31-32
- **PROTEIN KINASE STUDIES** 33-40
- **OTHER RELATED STUDIES** 41

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

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HE 20.3173/2:

REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES

1. BEHAVIOUR OF TWO SECOND MESSENGERS: CAMP AND CGMP IN LEUKOCYTES OF PERIPHERAL BLOOD OF NORMAL AND LEUKEMIC SUBJECTS.

Notario A, Petrucci E, Azzara A, Zocchi MT, Parversi I.
Clinica Medica Ia 'A. Ferrata', Policlinico San Matteo, 27100 Pavia, Italy
Haematologica (Pavia); 66(6):721-732 1981

Cyclic AMP (cAMP) and cyclic guanosine monophosphate (cGMP) were determined in the peripheral blood of 10 healthy controls, of 5 patients with chronic myeloid leukemia (CMA), of 5 with acute myeloblastic leukemia (AML), of 5 with chronic lymphatic leukemia (CLL), and of 5 with acute lymphoblastic leukemia (ALL), using a new radioimmunoassay. Comparing values of normal granulocytes with those for leukocytes of CML and AML, cAMP was decreased by 12% and 88%, respectively, and cGMP was decreased 34% and 62%, respectively. If the values of normal granulocytes, were compared with those for leukocytes of CLL and ALL, cAMP was decreased 68% and 59%, respectively, while cGMP was increased 24% in CLL, and decreased 23% in ALL. cAMP and cGMP were consistently decreased in acute leukemias (AML and ALL) and in CML. The ratio of cAMP/cGMP was similar for the two acute leukemias and quite different for the two chronic leukemias, especially for CLL. Possible causes for such behavior were considered. (52 Refs) (In ITA)

2. EFFECT OF MAGNESIUM CONTENT ON DENSITY-DEPENDENT REGULATION OF THE ONSET OF DNA SYNTHESIS IN TRANSFORMED 3T3 CELLS.

Rubin H
Dept. Molecular Biology, Univ. California, Berkeley, CA, 94720
Cancer Res; 42(5):1761-1768 1982

A spontaneously transformed clone of BALB/c 3T3 cells became more transformed after more than 90 passages as indicated by increased rounding of cells, multiplication to a higher saturation density, and increased ability to form colonies when suspended in agar. When the extracellular concentration of Mg++ was sharply reduced, the highly transformed cells flattened, assumed the shape of nontransformed cells, and became regularly arranged in cohesive arrays. If crowded when deprived of Mg++, they lost more intracellular Mg++ than did nontransformed and early passage-transformed cells and remained at constant cell density for at least 10 days. The intracellular content of neither Na+ nor K+ changed consistently, with Mg++ deprivation, but the Ca++ content increased more than two-fold. The sensitivity of the onset of DNA synthesis to inhibition by Mg++ deprivation increased with the extent of crowding of the cultures. This was demonstrated by varying population density within a single culture dish as well as from culture to culture. The loss of intracellular Mg++ in low concentrations of extracellular Mg++ increased with cell crowding as did the inhibition of DNA synthesis per fractional loss of intracellular Mg++. Neither deprivation of K+ or Ca++ nor addition of cyclic adenosine 3':5'-monophosphate produced a density-dependent inhibition of DNA synthesis. The results indicate that a reduction of the Mg++ content of highly transformed cells restores density-dependent inhibition of the onset of DNA synthesis, which is a characteristic property of nontransformed cells. The differences in Mg++ retentiveness with population density may reflect differences of intracellular distribution and binding of Mg+, which could in turn explain some of the regulatory effects of population density on metabolism and growth. (Author abstract) (16 Refs)

3. CAMP DIFFERENCES BETWEEN CLONES OF HIGH AND LOW MALIGNANT FIBROSARCOMA CELLS.

Elgebaly S, Kunkel S, Lovett EJ, Varani J
Dept. Pathology, Univ. Connecticut Health Center, Farmington, CT
Oncology; 39(3):163-166 1982

The levels of 3',5'-cyclic adenosine monophosphate (cAMP) were measured in a high malignant and a low malignant clone of murine fibrosarcoma cells isolated from a common parent and in normal mouse fibroblasts at various stages of growth. There was an inverse correlation between the degree of malignant potential and the cAMP levels. The normal fibroblasts had the highest cAMP at all time periods measured. The high malignant cells had the lowest levels and the low malignant cells were intermediary. The degree of susceptibility of each population to the growth inhibiting effects of dibutyryl cAMP, a cAMP analogue, was also measured. There was a direct correlation between degree of malignant potential and susceptibility to growth inhibition. These findings strongly support our belief that the low malignant cells represent a state of malignancy that is intermediary between the high malignant cells and the normal fibroblasts. cAMP differences may contribute to this. (Author abstract) (14 Refs)

4. THE RELATION BETWEEN CELL PROLIFERATION AND ADENYLATE CYCLASE ACTIVITY IN GRAFTS OF 3-METHYLCHOLANTHRENE INDUCED MOUSE UTERINE CERVICAL TUMORS.

Kvinnsland S, Lonning PE, Forsberg JG
Dept. Anatomy, Univ. Lund, Biskopsgatan 7, S-223 62 Lund, Sweden
Experientia; 38(3):387-389 1982

In transplanted uterine cervical tumors, induced by 3-methylcholanthrene, a significant negative correlation was obtained between adenylate cyclase activity and cell proliferation. (Author abstract) (17 Refs)

5. USE OF CYCLIC ADENOSINE-3'5'-MONOPHOSPHATE AND ITS ANALOGS IN CANCER CHEMOTHERAPY.

Riazanov EM
N. Petrov Res. Inst. Oncology, Leningrad, USSR
Eksp Onkol; 4(1):8-13 1982

Literature data pertaining to the applications of cyclic AMP (cAMP) and its analogs in experimental cancer chemotherapy are reviewed. Biological role of cAMP includes mediation of the proliferation and differentiation of normal and transformed cells. The content of cAMP in cultured tumor cells was shown to be lower than that in the normal cells. In vivo studies showed an increase of cAMP content in the adrenal cortex adenomas and hyperplasias, as compared with the normal adrenals. In vitro studies indicated that cAMP and its analogs inhibited the growth of cultured cells. The normal cells were less sensitive to cAMP than the homologous normal cells. The degree of inhibition depended upon the histology of tumor cells and the activity of protein kinases (PK). Treatment with chlorambucil or melphalan enhanced the activity of PK and increased the content of cAMP in sensitive Walker's carcinosarcoma and HeLa cells. Carminomycin did not affect cAMP level in murine lymphoid leukemia cells. (71 Refs) (In RUS)

6. CYCLIC NUCLEOTIDES IN CEREBROSPINAL FLUID OF PATIENTS WITH INTRACRANIAL AND SPINAL TUMORS.

Cramer H, Schindler E

Dept. Clinical Neurology, Univ. Freiburg, Hansastrasse 9, D-7800 Freiburg, W. Germany

Acta Neurol Scand; 65(3):174-181 1982

Concentrations of cyclic AMP (cAMP) and cyclic guanosine monophosphate (cGMP) in the cerebrospinal fluid (CSF) were studied in 47 adults (26 men, 21 women): 27 patients with peripheral nervous diseases not involving intraspinal structures (controls), and 20 patients with CNS tumors. In the controls, no significant correlation was observed between CSF cyclic nucleotide (CN) concentrations and age, sex, or CSF protein levels. The av cAMP/cGMP ratio in the CSF was 8.9:1. No correlation was observed between CSF levels of cAMP and cGMP. In patients with benign brain tumors (7/20), malignant brain tumors (8/20; primary in 4/8, metastatic carcinomas in 4/8), or malignant spinal cord tumors (5/20; primary plasmacytoma in 1/5, metastatic carcinomas in 4/5), a positive correlation was observed between CSF levels of cAMP and cGMP. The CSF CN concentrations were normal in the spinal tumor patients. A negative correlation was observed between CSF total protein and cAMP levels in the patients with malignant brain tumors. All but one of these patients underwent one lumbar puncture. The exception was a 16-yr-old patient with a meningeal sarcoma arising in the brain. The tumor later extended to involve the spinal subarachnoid space and spinal nerve roots; two lumbar punctures were performed when signs of progressive disease were evident. In this patient, the CSF cAMP concentration increased markedly (0.7-80.9 picomoles/ml) as the tumor extended to the spinal meningeal space, suggesting massive secretion of cAMP by the tumor cells. (18 Refs)

7. RECENT PROGRESS IN ANTINEOPLASTIC DRUG RESEARCH IN CHINA.

Xu B

Shanghai Inst. Materia Medica, Chinese Acad. Sciences, Shanghai, People's Republc of China

Cancer Research in the People's Republic of China and the United States of America: Epidemiology, Causation and New Approaches to Therapy, Proceedings of a Conference held in New York, 28-29, March, 1980. Marks PA, ed. New York, Grune and Stratton, 273 pp., 1981.

Agents which have potential anticancer activity are summarized. Agents discussed are methoxysarcolysin, N-formyl-sarcolysin, nitrocaphane (AT-1258), tisupurine (AT-1438), antimony-71, camptothecin, 10-hydroxycamptothecin, harringtonine, homoharringtonine, colchicine, colchicinamide, curzerenone, monocrotaline, lycobetaine, oridonin, indirubin, peiyanmycin, and oxalysine. Some derivatives of cantharidin, hydroxycantharidinamine, methylcantharidin, and sodium cantharidin have been investigated and found to have antitumor activity. In China some new types of anticancer agents have attracted attention, including tilorone, polyinosinic acid-polycytidylic acid, interferon-inducers, bromo-cyclicAMP, and poly-saccharides from Bacterium prodigiosum and mushrooms; some of the agents are in clinical trial. (36 Refs)

8. CURRENT CANCER RESEARCH ON CELL DIFFERENTIATION, TUMOR GROWTH, INVASION, AND METASTASIS (SPECIAL LISTING).

International Cancer Research Data Bank Program Office

NCI, Westwood Building, Room 10A18, Bethesda, MD, 20205

Cell Differentiation, Tumor Growth, Invasion, and Metastasis (Special Listing) This Title is Available Through National Technical Information Service, Springfield, VA, as NTISUB/PB81-929903;; 76pp. 1981

Special Listings of Current Cancer Research Projects are a service of the International Cancer Research Data Bank (ICRDB) program of the National Cancer Institute. Each listing contains descriptions of ongoing projects in one selected cancer research area. The descriptions are provided by cancer scientists in about 50 different countries. Regulation of tumor cell differentiation and growth is discussed with respect to genetic and epigenetic mechanisms, cell surface components and membrane function, hormones and other factors, cyclic nucleotides, and polyamine regulation of growth. Differentiation and growth in normal and leukemic hematopoietic cell differentiation and growth (differentiation of hematopoietic stem cells, RBC, and lymphocytes) and in embryonal carcinoma cells is reviewed. Cell differentiation and proliferation is described. Mechanisms and characteristics of tumor vascularization and invasion and metastases are outlined, including the role of enzymes in invasion and metastasis (clotting and fibrinolytic enzymes, collagenolytic enzymes, and other proteolytic enzymes and destructive substances in tumor invasion and growth), cell surface components and cell-cell interactions, immunological mechanisms involved in metastases, chemotaxis and locomotion of tumor cells, and other studies.

REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES

9. GENETIC ANALYSIS OF THE ROLE OF CAMP IN MEDIATING EFFECTS OF INTERFERON.

Schneck J, Rager-Zisman B, Rosen OM, Bloom BR

Dept. Microbiology and Immunology, Albert Einstein Coll. Medicine, Bronx, NY, 10461

Proc Natl Acad Sci USA; 79(6,part1):1879-1883 1982

The effects of interferon (IFN) on Fc receptor-mediated phagocytosis, intracellular cAMP levels, antiviral activity, and growth inhibition were analyzed in a cloned macrophage-like cell line, J774.2, and variants derived from it. Purified IFN increased Fc receptor-mediated phagocytosis in J774.2 cells, and in cAMP-responsive nonphagocytic variants but was without effect in cAMP-unresponsive nonphagocytic variants, in adenylate cyclase-deficient variants, and in cAMP-dependent protein kinase-deficient variants. Under conditions in which IFN augmented phagocytosis, it increased intracellular levels of cAMP. Parental cells were highly sensitive to IFN-mediated growth inhibition. In contrast, cAMP-dependent protein kinase-deficient variants were only 1/100th as sensitive to growth inhibition by IFN. All cell lines tested, both responsive and unresponsive to cAMP, were equally protected by IFN against infection with vesicular stomatitis virus, demonstrating that the antiviral state was independent of cAMP. These results indicate that, in transformed macrophages, stimulation of phagocytosis and inhibition of growth by IFN are mediated through intracellular cAMP, whereas the antiviral state induced by IFN is independent of cAMP. (Author abstract) (31 Refs)

10. POSITIVE AND NEGATIVE CAMP-MEDIATED CONTROL OF TYROSINE AMINOTRANSFERASE SYNTHESIS IN REUBER H35 HEPATOMA CELLS.

Snoek GT, Voorma HO, Van Wijk R
Laboratorium voor Moleculaire Celbiologie der Rijksuniversiteit te, Utrecht, Transitorium 3 Universiteits Centrum 'De Uithof', Padualaan 8, NL-3584-CH Utrecht, The Netherlands
Eur J Biochem; 123(1):217-222 1982

Induction of L-tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5) by 6,O-dibutyryl-adenosine 3',5'-monophosphate (Bt2cAMP) in Reuber H35 hepatoma cells reaches a maximum value between 3-5 hr after addition of Bt2cAMP and subsequently decreases in the continuous presence of BtcAMP. We have investigated the kinetics of the increase, ie, induction, and the decrease, ie the repressed state, of the tyrosine-aminotransferase-synthesizing system under these conditions. Our experimental results are as follows. (1) The repressed state of the tyrosine-aminotransferase-synthesizing system is not caused by a decrease in the intracellular cAMP concentration. (2) The repressed state is inhibited by actinomycin D (while induction is not inhibited). (3) During the repressed state no effect of dexamethasone on tyrosine aminotransferase synthesis is found, while during induction Bt2cAMP and dexamethasone act synergistically. (4) Longer starvation of the cells in serum-free medium has no influence on the kinetics of the induction/repressed state curve. From these results we have concluded that the mechanism of the transition to the repressed state of the tyrosine-aminotransferase-synthesizing system is essentially different from the mechanism of deinduction which occurs after removal of the inducer. Moreover, the repressed state of the system is a phenomenon which is induced by Bt2cAMP separately from induction at a different level of protein synthesis. (Author abstract) (39 Refs)

11. INVERSE CORRELATION BETWEEN CYCLIC GMP AND TYROSINE AMINOTRANSFERASE DEGRADATION IN RAT HEPATOMA TISSUE CULTURE CELLS.

Strinden ST, Stellwagen R
Dept. Obstetrics and Gynecology, Univ. Wisconsin, Madison, WI, 53705
Biochem Biophys Res Commun; 105(2):724-729 1982

The effects of insulin and cyclic nucleotide phosphodiesterase inhibitors (1-methyl-3-isobutylxanthine, papaverine, theophylline, caffeine, theobromine) on cGMP and cAMP levels in rat hepatoma tissue culture (HTC) cells were investigated to determine whether they produce changes in cyclic nucleotide levels which are correlated with tyrosine aminotransferase (TAT) degradation. All five phosphodiesterase inhibitors decreased cGMP levels, while the effects on cAMP levels were variable. This lowering effect on cGMP may be due to the fact that the phosphodiesterase inhibitors also inhibit guanylate cyclase. Since previous studies have shown that the rate of TAT degradation is increased in the presence of phosphodiesterase inhibitors, these results indicate an inverse correlation between cGMP levels and TAT degradation; there does not appear to be any correlation between cAMP and TAT degradation. Insulin increased the cGMP level in HTC cells. Under the same conditions insulin slowed the degradation of TAT and reversed the effect of theophylline on TAT degradation. This suggests that the effect of insulin on TAT degradation may be mediated, at least partially, by an increase in cGMP. (20 Refs)

12. PARADOXICAL EFFECTS OF GLUCOCORTICOIDS ON REGULATION OF PLASMINOGEN ACTIVATOR ACTIVITY OF RAT HEPATOMA CELLS.

Barouski-Miller PA, Gelehrter TD
Dept. Human Genetics and Internal Medicine, Univ. Michigan Medical Sch., Ann Arbor, MI, 48109
Proc Natl Acad Sci USA; 79(7):2319-2322 1982

Incubation of rat hepatoma cells with cAMP derivatives stimulates cell-associated plasminogen activator activity 8-to 22-fold and extracellular plasminogen activator activity 30-to 1,300-fold. This time- and concentration-dependent increase is enhanced by phosphodiesterase inhibitors. Dexamethasone, a synthetic glucocorticoid, decreases the plasminogen activator activity of these cells, probably through induction of an inhibitor. Paradoxically, dexamethasone, added simultaneously with cAMP derivatives causes a further four-fold enhancement of the cAMP-mediated stimulation of plasminogen activator activity. Dexamethasone also alters the time course of cAMP-mediated enhancement of plasminogen activator activity: increased protease activity is detected at 4 hr in cells incubated with 8-bromoadenosine-3':5'-cyclic monophosphoric acid and 1-methyl-3-isobutylxanthine but not until 12 hr in cells incubated with dexamethasone as well. Glucocorticoids thus exert two separate and opposite effects on plasminogen activator activity: induction of an inhibitor and amplification of cyclic nucleotide action. Although permissive and synergistic effects of dexamethasone on cyclic nucleotide action have been reported previously, glucocorticoid regulation of plasminogen activator activity is unique in that the amplification of cyclic nucleotide effects by dexamethasone opposes its regulatory action toward a specific enzyme. (Author abstract) (35 Refs)

13. PROLACTIN STIMULATION OF ORNITHINE DECARBOXYLASE ACTIVITY IN CULTURED RAT MAMMARY TUMORS.

Frazier RP, Costlow ME
Dept. Biochemistry, St. Jude Children's Res. Hosp., Memphis, TN, 38101
Exp Cell Res; 138(1):39-45 1982

Ovine prolactin increased ornithine decarboxylase (ODC) activity in 13 of 14 primary cultures of 7,12-dimethylbenzanthracene-induced rat mammary tumors. The 27-436% increase (mean 219%) in ODC activity required a 3-hr incubation with at least 1,000 ng prolactin/ml. Lower prolactin concentrations were ineffective. Bovine fetal serum or other hormones were not required for the prolactin effect. Addition of dibuturyl cyclic AMP (db-cAMP) (2.5 mM) to cultures increased ODC activity two-fold, whereas dibuturyl cyclic GMP (db-cGMP) (2.5 mM) had little effect. When either nucleotide plus 10 or 100 ng prolactin/ml was added. ODC activity increased three- to four-fold. These results show that increased ODC activity is an early event in prolactin action in cultured rat mammary tumor cells. The cyclic nucleotides appear to be necessary for the cells to respond to physiologic levels of hormone. (Author abstract) (33 Refs)

14. RESTORATION BY CYCLIC AMP OF THE DIFFERENTIATED PHENOTYPE OF CHONDROCYTES FROM DEDIFFERENTIATED CELLS PRETREATED WITH RETINOIDS.

Takigawa M, Takano T, Suzuki F
(c/o Suzuki) Dept. Biochemistry and Calcified-Tissue Metabolism., Osaka Univ., 3-48, Nakanoshima 4-Chome, Kita-Ku, Osaka 530, Japan
Mol Cell Biochem; 42(3):145-153 1982

Parathyroid hormone (PTH) increases the cyclic AMP level in rabbit costal chondrocytes in culture. PTH, dibutyryl cyclic AMP (DBcAMP), and 8-bromo cyclic AMP (8-Br cAMP) induce ornithine decarboxylase (ODC) and expression of the differentiated phenotype of chondrocytes in this cell system. On the other hand, retinoids inhibit expression of the differentiated phenotype of chondrocytes. In the present study, the effects of PTH, DBcAMP, and 8-Br cAMP on rabbit costal chondrocytes pretreated with retinoids were examined. PTH did not increase the cellular cyclic AMP level in de-differentiated cells that had been pretreated with retinyl acetate or retinoic acid for 3 days, but it did increase the cyclic AMP level 4 days after removal of retinoids. PTH did not stimulate ODC activity or expression of the differentiated phenotype of chondrocytes in the de-differentiated state. On the other hand, DBcAMP or 8-Br cAMP stimulated expression of the differentiated phenotype of chondrocytes even in de-differentiated cells, as judged by morphological and histological changes of the cells and increase in glycosaminoglycan synthesis. Cyclic AMP analogues also induced ODC in these cells. (Author abstract) (34 Refs)

15. DECREASED SYNTHESIS OF LARGE FUCOSYL GLYCOPEPTIDES DURING DIFFERENTIATION OF EMBRYONAL CARCINOMA CELLS INDUCED BY RETINOIC ACID AND DIBUTYRYL CYCLIC AMP.

Muramatsu H, Muramatsu T
Dept. Biochemistry, Kagoshima Univ. Sch. Medicine, Usukicho 1208-1, Kagoshima 890, Japan
Dev Biol; 90(2):441-444 1982

The F9 clone of a teratocarcinoma stem cell line (EC) was induced to differentiate in vitro by retinoic acid (1 uM) and dibutyryl cyclic AMP (1 milliM). Synthesis of large fucosyl glycopeptides (belonging to the lactosaminoglycan series, with a galactose and N-acetylglucosamine core structure) declined as the F9 cells differentiated morphologically. The amount of fucose in the particulate fraction of cells (calculated from the protein content) declined slightly during cell differentiation. The differentiation system of F9 cells, with a clear-cut change in the high-mol wt carbohydrate profile during differentiation, is a suitable model for further study of the causal relationship between altered glycosylation and induction of cell differentiation. (14 Refs)

16. DEPOSITION OF BASEMENT MEMBRANE PROTEINS IN ATTACHMENT AND NEURITE FORMATION OF CULTURED MURINE C-1300 NEUROBLASTOMA CELLS.

Alitalo K, Kurkinen M, Virtanen I, Mellstrom K, Vaheri A
Dept. Virology, Univ. Helsinki, Haartmaninkatu 3, SF-00290 Helsinki 29, Finland
J Cell Biochem; 18(1):25-35 1982

The deposition of basement membrane (BM) glycoproteins during the processes of substrate attachment and cell differentiation was studied in cultures of C-1300 mouse neuroblastoma cells (clone NB41A3). Cell differentiation was induced by sparse plating and the addition of dibutyryl cyclic AMP (dbcAMP; 1 milliM) to the medium. Indirect immunofluorescence microscopy disclosed a typical perinuclear cytoplasmic staining for BM antigens in both differentiated and undifferentiated cells. No surface fluorescence was detected in freshly seeded cells in suspension, but punctate deposits of laminin were detected on the ventral surfaces of the cells within 2 hr after plating. In sparsely seeded cultures of undifferentiated C-1300 cells, extracellular laminin deposits were detected only under the primary attaching cells, not the daughter cells in the clonal colonies. In dbcAMP-exposed cultures, laminin deposition was detected in association with the growing cytoplasmic extensions of the newly formed neurites. The secreted proteins of cultured differentiated and undifferentiated neuroblastoma cells showed markedly different patterns of fucosylation of the high-mol wt proteins. No deposits of fibronectin or Type IV collagen were found under the attaching and a differentiating cells. It is concluded that cultured C-1300 cells can synthesize, secrete, and deposit laminin. The distribution of laminin during cell attachment and neurite extension suggests that laminin may be involved in cell-to-substrate interactions in cultured C-1300 neuroblastoma cells. (53 Refs)

17. DOME FORMATION BY A RETROVIRUS-INDUCED LUNG ADENOCARCINOMA CELL LINE.

Rizzino A, Gonda MA, Rapp UR
(c/o Rapp) Lab. Viral Carcinogenesis, NCI, Frederick, MD, 21701
Cancer Res; 42(5):1881-1887 1982

A cell line has been established from an alveologenic lung adenocarcinoma that develops in newborn NFS/N mice after ip inoculation with a carcinoma-inducing retrovirus. A clonal derivative of this cell line, 3041, forms adenocarcinomas when injected into syngeneic weanling mice and in culture retains several properties typical of alveologenic Type 1 cells. These include morphological details, such as tight junctions and interdigitating microvilli in the areas of cell-cell contact, as well as the ability to transport fluid in culture. Fluid transport becomes evident in confluent cultures by the formation of domes or hemicysts. The formation of domes, which is considered to reflect a differentiated function of these secretory epithelial cells, can be enhanced more than 50-fold by treatment with dibutyryl cyclic adenosine 3':5'-monophosphate but only 5-and 3-fold by sodium n-butyrate and dimethylsulfoxide, respectively. It is anticipated that the secretory alveologenic cell line 3041 described here will be useful for studies of both its pathological and its physiological properties. (Author abstract) (23 Refs)

18. REGULATION OF DIHYDROFOLATE REDUCTASE GENE TRANSCRIPTION IN METHOTREXATE-RESISTANT MOUSE FIBROBLASTS.

Wu JS, Johnson LF
(c/o Johnson) Dept. Biochemistry, Ohio State Univ., Columbus, OH, 43210
J Cell Physiol; 110(2):183-189 1982

DNA-excess filter hybridization was used to study the rate of synthesis of dihydrofolate reductase (DHFR) heterogeneous nuclear (hn)RNA in serum-stimulated M50L3 cells, a methotrexate-resistant mouse 3T6 cell line. The rate of synthesis of DHFR hnRNA relative to total hnRNA varied over a considerable range when resting M50L3 cells were induced to reenter the cell cycle. The percentage of total hnRNA synthesized during a 20-min labeling period that hybridized to DHFR complementary (c)DNA was about 0.02-0.03% in resting

M50L3 cells. When resting cells were serum stimulated to reenter the cell cycle, the percentage decreased initially to approx 0.01%, then began increasing about 6 hr following stimulation, and reached a max of approx 0.08% by approx 15 hr after stimulation. The relative rate of DHFR hnRNA synthesis began increasing at approx the same time (6 hr) and increased to approx the same extent by 15 hr following stimulation (3-4-fold) as does the relative rate of cytoplasmic DHFR mRNA. It was suggested that changes in the relative rate are primarily due to changes in the transcription of the DHFR gene. However, it is possible that the rate of synthesis of DHFR hnRNA remains relatively constant but that changes in the rate of synthesis of total hnRNA are responsible for alterations in the relative rate of DHFR hnRNA synthesis. The intracellular signals responsible for controlling DHFR gene expression are not known. High intracellular cyclic AMP was able to suppress DHFR gene transcription even when progression through the cell cycle was not blocked. The relationship of DHFR gene transcription to entry into the S phase was examined. (19 Refs)

19. REGULATION OF AMINO ACID TRANSPORT INTO EHRLICH CELLS. EFFECT OF 3',5'-ADENOSINE MONOPHOSPHATE ON L-LEUCINE TRANSPORT.

Cuesta Casado MC, Ramos EH
Instituto de Quimica Biologica, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, Argentina
Cell Mol Biol; 27(6):635-641 1981

The effect of adenosine 3',5'-cyclic monophosphate on L-leucine transport was studied regarding its regulatory action on one parameter under pleiotypic control. The metabolic processes related to biosynthesis and degradation of cAMP operate in Ehrlich cells. An increase in the intracellular content of the cyclic nucleotide partially inhibits the transport of L-leucine. The regulatory effect is maintained in all stages of tumor growth. In vitro experiments showed no effect of cAMP or Bu2cAMP on (Na+ - K+)-ATP-A activity of plasma membranes under the same experimental conditions as in in vivo experiments. (Author abstract) (20 Refs)

20. EFFECTORS OF FATTY ACID SYNTHESIS IN HEPATOMA TISSUE CULTURE CELLS.

Mapes JP, Aultman KS
Dept. Biochemistry, Louisianna State Univ. Medical Center, New Orleans, LA, 70112
Arch Biochem Biophys; 214(2):782-791 1982

The importance of substrates and the effects of cyclic AMP, fatty acids, ammonium ion, and glutamine on lipogenesis in hepatoma tissue culture (HTC) cells were investigated and compared to findings with normal liver cells. Fatty acid synthesis in HTC cells depended on the addition of the substrate, with the order of stimulation being glucose + pyruvate approx = glucose + alanine approx = glucose + lactate approx = pyruvate greater than glucose greater than alanine much greater than no additions. Oleate decreased fatty acid synthesis. In contrast to normal liver tissue, dibutyryl-cyclic AMP inhibited neither glycolysis nor fatty acid synthesis. Stimulators of the hepatic malate-aspartate shuttle in normal liver had mixed effects on the redox state and fatty acid synthesis in HTC cells; ammonium ion and glutamine decreased the redox potential and increased the rate of fatty acid synthesis, while lysine did not affect either process. Other possibilities of stimulation of fatty acid synthesis were investigated. It was concluded that increased glycolysis in HTC cells explains the stimulation of fatty acid synthesis by NH4+ and glutamine, even though glycolysis is much more rapid than fatty acid synthesis in these cells. This conclusion was supported by the observation that the percentage increase in glycolysis caused by NH4+ or glutamine closely matched the percentage increase in fatty acid synthesis and that the malate-aspartate shuttle, the pentose phosphate pathway, and the steps past pyruvate were not limiting in the absence of NH4+ or glutamine. (31 Refs)

21. STUDIES ON THE MECHANISM OF NK CELL LYSIS.

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Dept. Microbiology and Immunology, Albert Einstein Coll. Medicine, Bronx, NY

J Immunol; 128(4):1786-1791 1982

The mechanism of cytolysis by murine NK cells was analyzed using a variety of metabolic inhibitors that have proven informative in studying the lytic mechanism of CTL and the mechanism of histamine release by mast cells. Target cell binding occurred in the absence of calcium and was inhibited by only one of the agents studied, cytochalasin B. Lysis was initiated by addition of Ca++ ions, as in the case of CTL. Subsequent to target cell binding, but prior to programming for lysis by Ca++, NK cell lytic activity could be suppressed by inhibitors of chymotrypsin-like, but not trypsin-like proteases, in contrast to CTL. In addition, 3-deaza-SIBA, an inhibitor of transmethylation reactions and quinacrine, an inhibitor of phospholipase A2, appear to act before the Ca++-dependent programming for lysis. Sr++ ions blocked the lytic function, as did trifluoperazine (stelazine), the former presumably competing for ionic calcium, the latter known to block binding of Ca++ to calmodulin. 8Br-cAMP and colchicine blocked later steps required for lysis. With the possible exception of trifluoperazine, all of the agents that blocked NK cell lysis are known to inhibit histamine release from mast cells. These results lend support to the stimulus-secretion model, originally proposed to explain the mechanism of CTL cytolysis, as relevant to the mechanism of lysis by NK cells. (Author abstract) (38 Refs)

CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES

22. PARTIAL PURIFICATION FROM HEPATOMA CELLS OF AN INTRACELLULAR SUBSTANCE WHICH MEDIATES THE EFFECTS OF INSULIN ON PYRUVATE DEHYDROGENASE AND LOW KM CYCLIC AMP PHOSPHODIESTERASE.

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Arch Biochem Biophys; 215(1):339-344 1982

An enzyme-stimulating factor was isolated from hepatoma cells. The factor stimulated pyruvate dehydrogenase (PD) and low Km cyclic AMP phosphodiesterase in a dose-dependent fashion. PD stimulation was sodium-sensitive. The factor was similar to the second messenger of insulin derived from other cell types in acid stability, mol wt, insulin-dependent generation, and elution from the anion-exchange resin AG 1-X8 at an ionic strength of 0.4 M. (21 Refs)

23. RECEPTOR-ASSOCIATED CHANGES OF THE CATECHOLAMINE-SENSITIVE ADENYLATE CYCLASE IN GLIOMA CELLS DOUBLY TRANSFORMED WITH MOLONEY SARCOMA VIRUS.

Higashida H, Miki N, Tanaka T, Kato K, Nakano T, Nagatsu T, Kano-Tanaka K
Cancer Res. Inst., Kanazawa Univ., Kanazawa 920, Japan
J Cell Physiol; 110(2):107-113 1982

Activity of adenylate cyclase (AC) and the receptor binding assay of 3H-dihydroalprenolol (DHA) for the beta-adrenergic receptor of C6 glioma cells doubly transformed with Moloney sarcoma virus (C6V-1 cells) were carried out as a model system for viral modification of the plasma membrane and compared with those of C6 cells. Intracellular cyclic AMP levels and intracellular phosphodiesterase, mitochondrial monoamine oxidase, and cytosomal enolase activities were measured in both cell lines. C6V-1 cells differed from C6 cells morphologically. C6V-1 cells were composed of large numbers of both round and fusiform cells, whereas C6 cells contained predominantly fusiform cells. C6V-1 cells grew faster than C6 cells with mean doubling times of 17 and 22 hr, respectively. The basal activity of AC was always lower in C6V-1 cells than in C6. Effects of Mg++, prostaglandin E1, isoproterenol, and norepinephrine on AC activity were determined in both cell lines. Fewer DHA binding sites were observed in C6V-1 cells than in C6 cells. Intracellular cyclic AMP content in C6 and C6V-1 cells was 10.1 and 4.4 picomoles/mg protein, respectively. Phosphodiesterase activity in C6 cells was 3x greater than that in C6V-1 cells, with mean activities of 91 and 31 picomoles/mg protein, respectively. Both cell types showed type A mitochondrial monoamine oxidase activity in the range of approx 70-90 picomoles/min/mg protein. Enolase activity was higher in C6V-1 cells than in C6 cells, with specific activities of 1.6 and 2.3 units/mg protein, respectively. (30 Refs)

24. RECEPTORS FOR REGULATORY PEPTIDES IN EPITHELIAL GUT CANCER.

Forgue-Lafitte ME, Laburthe M, Zweibaum A, Rosselin G
INSERM U.55, Hopital Saint-Antoine, Paris 12, France
Protides Biol Fluid Proc Colloq; 29:595-598 1982

Studies on receptors for regulatory peptides, such as insulin and vasoactive intestinal peptide (VIP) in transformed gut epithelial cell lines from humans are reviewed. The coupling of insulin and VIP with their specific receptors triggers regulatory events: insulin regulates long-term effects (protein, RNA, and DNA synthesis), while VIP regulates events leading to glycogenolysis by activation of adenylate cyclase, cyclic AMP-dependent protein kinases, and phosphorylase a. Insulin is effective at very low concentration (10(-10) M). It was demonstrated that transformed cells of the digestive tract possess specific receptors for regulatory peptides which make them sensitive to their neuroendocrine environment. The insulin effect was not uniform; even in the same type of cell, the effect on growth can vary according to the batch of serum used. These receptors are coupled to a biological activity, the magnitude of which varies according to the cell line. (30 Refs)

25. DESENSITIZATION OF TUMOUR LEYDIG CELLS BY LUTROPIN: EVIDENCE FOR UNCOUPLING OF THE LUTROPIN RECEPTOR FROM THE GUANINE NUCLEOTIDE-BINDING PROTEIN.

Dix CJ, Schumacher M, Cooke BA
Dept. Biochemistry and Chemistry, Royal Free Hosp. Sch. of Medicine, Univ. London, 8 Hunter St., London WC1N 1BP, England
Biochem J; 202(3):739-745 1982

Purified rat Leydig tumors cells were pretreated with lutropin and the effect on the subsequent response to lutropin was determined. Maximal cyclic AMP production was achieved with the same concentration of lutropin in control and lutropin-pretreated cells; however, the maximum stimulated level in pretreated cells was only 30% of controls. The sensitivity to lutropin was decreased in lutropin-pretreated cells, as was the rate of maximal cyclic AMP production. However, cholera-toxin-stimulated cyclic AMP production was not decreased by lutropin pretreatment, and a potentiation was seen at all time points studied. Pre-incubation with lutropin caused a decrease in specific 125I-labeled human choriogonadotropin binding; however, this decrease was abolished if the cells were washed under acidic conditions, indicating that occupation but not loss of the lutropin receptors had taken place. THe effect of pretreating the cells with lutropin on adenylate cyclase activity in purified plasma membranes was also investigated. In plasma membranes from control cells both guanosine 5'-[beta-gamma-imido]triphosphate [p(NH)ppG] plus lutropin and NaF plus lutropin caused a 50-60-fold linear increase in cyclic AMP production over 40 min compared with 15-fold with p(NH)ppG and 6-fold with lutropin alone. In plasma membranes isolated from lutropin-treated cells the NaF-plus-lutropin- and the p(NH)ppG-stimulated cyclic AMP production rates were unchanged but no effect of lutropin could be demonstrated with or without added p(NH)ppG. In contrast the plasma membranes from dibutyryl cyclic AMP-treated cells had similar cyclic AMP production rates to control cells with all stimulants studied. The present evidence obtained from studies both with intact cells and with isolated plasma membranes indicates that the initial lutropin-induced desensitization of the rat Leydig tumor cell is due to a lesion in the hormone-receptor coupling to the guanine nucleotide regulatory protein. This process is apparently not regulated by cyclic AMP. (33 Refs)

26. ULTRACYTOCHEMICAL LOCALIZATION OF ESTROGEN-STIMULATED GUANYLATE CYCLASE IN RAT UTERUS.

Kang YH, Sahai A, Criss WE, West WL
Dept. Pathology, USUHS, Bethesda, MD, 20814
J Histochem Cytochem; 30(4):331-342 1982

Estrogens are known to increase cyclic guanosine monophosphate (cGMP) levels in the uterus of rats by enhancing guanylate cyclase (GC) activity. In the present study, the cytochemical localization of GC activity was studied in the uteri of immature and ovariectomized rats after treatment with diethylstilbestrol (DES), progesterone, estrogen antagonist (C1628), and a combination of DES and C1628. Twenty-four hr after the first dose of DES, moderate to strong guanylate cyclase activity was indicated by lead phosphate precipitate on the luminal microvillar and basolateral surfaces of epithelial cells, whereas strong activity was found on the plasma membranes of fibroblasts, endothelial cells, and myometrial cells. The enzyme activity in the epithelial cells declined slightly 24 hr after the second daily dose of DES. Uterine tissues from DES-treated rats that were preheated at 60 C for 30 min or preincubated with a GC inhibitor showed no reaction product. Guanylate cyclase activity was not observed cytochemically in the uterine tissues of the vehicle control (immature or ovariectomized) or progesterone- and C1628-treated animals. Weak guanylate cyclase activity was observed on the plasma membranes of epithelial cells and endothelial cells after doses of DES and C1628 were given simultaneously. The biochemical assays of the total homogenate in vitro indicated that uterine GC showed about a twofold increase after one dose of DES and a 1.3-fold increase following two doses (1 dose per day) of DES when compared with their respective nontreated controls, or with progesterone-treated uteri. GC was found in particulate (90%) and cytosol (10%) fractions. These data demonstrated that DES stimulated uterine guanylate cyclase activity, while progesterone and C1628 were ineffective at the doses used. Estrogen antagonist C1628 does not completely suppress the effect of DES. (Author abstract) (38 Refs)

27. [HALF-CYS4,HALF-CYS(10)]-ALPHA-MELANOCYTE-STIMULATING HORMONE: A CYCLIC ALPHA-MELANOTROPIN EXHIBITING SUPERAGONIST BIOLOGICAL ACTIVITY.

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Proc Natl Acad Sci USA; 79(6,part1):1751-1755 1982

Alpha-Melanocyte-stimulating hormone (alpha-melanotropin; alpha-MSH) is a linear tridecapeptide (Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val NH2) that reversibly darkens amphibian skins by stimulating melanosome (pigment granule) dispersion within melanophores. By using a number of in vitro melanocyte assays, we have examined the conformational requirements for alpha-MSH activity. Synthesis of [half-Cys4,half-Cys(10)]-alpha-MSH, a cyclic, conformationally restricted 'isoteric' analogue of alpha-MSH, provides a melanotropin with a potency greater than 10,000x that of the native hormone in stimulating frog (Rana pipiens) skin darkening. The cyclic analogue also showed substantially prolonged activity relative to the native hormone. [half-Cys4,half-Cys(10)]-alpha-MSH was approx 30x more potent than alpha-MSH in stimulating lizard (Anolis carolinensis) skin melanophores in vitro. By using a cell-free Cloudman S-91 mouse melanoma plasma membrane preparation, we found the cyclic analogue to be approx 3x as potent as the native hormone in stimulating adenylate cyclase activity. These results provide insight into the conformational requirements for biological activity of alpha-MSH, and the comparative conformational requirements of alpha-MSH at a number of pigment cell receptors. (Author abstract) (28 Refs)

28. LACK OF FUNCTION OF HISTAMINE H1 AND MUSCARINIC ACETYLCHOLINE RECEPTORS OF MOUSE NEUROBLASTOMA CELLS GROWN IN SERUM-FREE MEDIUM.

Carlson J, Smith A, Richelson E
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In Vitro; 18(3):175-178 1982

Mouse neuroblastoma cells (Clone N1E-115) were grown in serum-free (defined) medium, defined medium supplemented with serum, and control medium to determine whether serum-free medium could substitute for serum-containing medium in our studies of the histamine H1 and muscarinic acetylcholine receptors of these cells. The function of these receptors as determined by measurements of receptor-mediated cyclic [3H]GMP formation was absent in cells grown in serum-free medium and increased as the percentage of serum was increased in the defined medium, but never attained the levels found with control cells. Muscarinic receptor number for cells grown in defined medium was 60% above that found for control cells with no change in the affinity of the receptor for the radioligand (-)[3H]quinuclidinyl benzilate. Guanylate cyclase and acetylcholinesterase activities for cells grown in defined medium were 23 and 66% of those found in control cells, respectively. This marked reduction of guanylate cyclase activity in large part explains the lack of function of these receptors. (Author abstract) (11 Refs)

29. DISORDERS OF CALCIUM AND MAGNESIUM HOMEOSTASIS.

Agus ZS, Wasserstein A, Goldfarb S
Hosp. Univ. Pennsylvania, 860 Gates Building, 3400 Spruce St., Philadelphia, PA, 19104
Am J Med; 72(3):473-488 1982

The components of calcium and magnesium balance and the factors responsible for the maintenance of the serum concentration of these cations are reviewed. Within this framework, the causes and treatment of disturbances of the serum concentration are discussed. Hypercalcemia is usually a reflection of increased bone resorption and/or gut absorption with the kidney playing a secondary role. Hypocalcemia is usually due to either a disturbance in the parathyroid hormone-adenylate cyclase system or a disturbance in vitamin D metabolism. As vitamin D is required for expression of the action of PTH at bone and as PTH is a prime regulator of vitamin D metabolism, the absence of either component results in important disturbances in calcium balance. In contrast to calcium homeostasis, the kidney plays a major role in the determination and regulation of serum magnesium. The major causes of hypermagnesemia therefore are associated with loss of renal function, and hypomagnesemia is frequently due to renal magnesium wasting. (Author abstract) (110 Refs)

CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS

30. MODULATION BY ISLET-ACTIVATING PROTEIN OF ADENYLATE CYCLASE ACTIVITY IN C6 GLIOMA CELLS.

Katada T, Amano T, Ui M
Dept. Physiological Chemistry, Hokkaido Univ., Sapporo 060, Japan
J Biol Chem; 257(7):3739-3746 1982

The cAMP content of intact cells as well as adenylate cyclase of the membrane-rich particulate fractions was studied with C6 glioma cells that had been exposed to the culture medium supplemented with islet-activating protein (IAP), one of the pertussis toxins. Both the increase in the cellular cAMP content in response to a beta-adrenergic agonist and the stimulation of membrane adenylate cyclase by the beta-agonist and/or GTP were markedly enhanced by the IAP treatment of C6 cells, but no change was induced in affinities of the agonist (or an antagonist) or GTP for their respective sites of action (or binding). The concentration of IAP required for the half-maximal enhancement was as low as 1 pg/ml, when the time of cell exposure to the toxin was prolonged to 18 hr. No enhancement was observed for the basal cAMP content or basal enzyme activity, nor was activation of adenylate cyclase by Gpp(NH)p (or NaF) affected by IAP treatment. The Vmax value of a specific and low Km GTPase was significantly smaller in the membranes of IAP-treated cells than in those of control cells. Cholera toxin treatment of cells activated adenylate cyclase without exerting any influence on these IAP actions. Thus, IAP would appear to enhance beta-receptor-coupled stimulation of adenylate cyclase, in a manner distinct from cholera toxin, by rendering more GTP available to the GTP sites in the regulatory subunit of the receptor-enzyme system. (Author abstract) (20 Refs)

CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES

31. IMPLEMENTATION OF MICROMETHODS TO RESOLVE PROBLEMS OF HUMAN BREAST TUMOR HETEROGENEITY IN ANALYSIS OF CYCLIC 3':5'-NUCLEOTIDE PHOSPHODIESTERASE.

Larner EH, Rutherford CL
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Cancer Res; 42(5):1661-1668 1982

Individual human infiltrating ductal carcinomas and fibroadenomas were sectioned and frozen to yield an alternating sequence of stained and lyophilized material. Stained preparations were used as references permitting microdissection of regions of tumor involvement in the corresponding dried sections. Tissue quantities of 5 to 25 ug dry weight were incubated under mineral oil in reaction volumes of 5 ul and analyzed for cyclic adenosine 3':5'-monophosphate phosphodiesterase (PDE). The observed affinity constants for the 27,000 x g soluble PDE from benign tumors were 4.7 and 49.9 uM, while those for malignant tumors were 6.3 and 28.5 uM. The soluble enzyme of both tumor types eluted in three peaks on DEAE-Sephacel microcolumns. Both tumor types possessed a PDE activator eluting at 350 mM NaCl, although endogenous PDE activities were unaffected by additions of either this activator or 200 uM ethylene glycol bis(beta-aminoethyl ether)N,N,N',N'-tetraacetic acid. Individual microsections of benign tumors contained total PDE levels two-fold higher than those of malignant tumors. Homogenates prepared from pooled microsections of the same tumors possessed only one-half of the total activity. Differential losses of enzyme in various preparation schemes as well as the use of tumor samples differing in cell density were suggested to account for some of the apparently conflicting literature values for breast tumor PDE. (Author abstract) (27 Refs)

32. BIOCHEMICAL MECHANISM OF RADIOPROTECTIVE ACTION OF ADENYL COMPOUNDS.

Koshcmeenko NN
No affiliation given
Biol Nauki; (2):5-18 1982

Literature data on the biochemical substrate of the radioprotective action of adenyl nucleotides (adenosine, adenine, and mixtures and complexes of adenylates with other radioprotective agents) are reviewed. The metabolism of the adenyl compounds, the hypotheses on their biologically active forms, and the hormone-like effect of adenosine are discussed. Adenosine may be bound to the adenylate cyclase receptors. (107 Refs) (In RUS)

PROTEIN KINASE STUDIES

33. RESISTANCE TO PHOSPHATASE OF THIOPHOSPHORYLATED EPIDERMAL GROWTH FACTOR RECEPTOR IN A431 MEMBRANES.

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Proc Natl Acad Sci USA; 79(7):2231-2235 1982

Epidermal growth factor (EGF) increases the phosphorylation of its receptor and other membrane proteins, and these proteins can be rapidly dephosphorylated by membrane-bound protein phosphatase. We report that [35S]adenosine 5'-[gamma-thio]triphosphate is as effective as [gamma-32P]ATP as substrate for the EGF receptor-associated protein kinase in A431 membranes. Both the kinetics and the extent of the EGF-dependent thiophosphorylation at 0 C are similar to those obtained with [gamma-32P]ATP, provided that ATP hydrolysis by the membrane preparation is inhibited by addition of adenosine 5'-[beta,gamma-imino]triphosphate. The thiophosphorylation reaction requires Mn + + but differs from the phosphorylation reaction in the inability of Mg + + to serve as cofactor. Both EGF-dependent phosphorylated and thiophosphorylated membrane proteins yield the same two major bands of Mr 145,000-160,000 in autoradiograms of Na-DodSO4/polyacrylamide gel electrophorograms. The rate of dephosphorylation of membrane proteins that have been thiophosphorylated in the presence of EGF is dramatically slower (factors of 1/20 to 1/40) than that of the phosphorylted proteins at both 0 C and 32 C. This increased metabolic stability of the thiophosphorylated proteins will be useful for investigation of the role of phosphorylation in the biological effects of EGF. (Author abstract) (22 Refs)

34. MICROSOMAL CAMP-INDEPENDENT HISTONE H1 KINASE ACTIVITY IN PLASMACYTOMA, MORRIS HEPATOMA AND NORMAL LIVER.

Schmitt M, Quirin-Stricker C, Kempf J

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Biochimie; 64(1):13-20 1982

A protein kinase activity with high specificity for histone H1 was isolated from mouse plasmacytoma, Morris hepatoma and normal mouse liver and compared by ion exchange chromatography after DEAE-cellulose, hydroxylapatite and Sephadex G-200 chromatography. This cAMP-independent histone H1 kinase is not affected by the heat-stable cAMP-dependent protein kinase inhibitor. It has the following particular properties: it prefers GTP to ATP as substrate and was found to be present with a great activity only in neoplastic tissues. No phosphatase activity was detected in the partially purified histone H1 kinase fraction from normal and neoplastic cells. These results suggest either an increased amount of histone H1 kinase and/or of its activator in neoplastic cells, or the presence of a strong inhibitor in normal cells. This histone H1 kinase appears to be analogous to the chromatin bound kinase which phosphorylates histone H1 at the NH2 and COOH terminal regions. We might suggest an implication of this kinase in the regulation of cell division. (Author abstract) (12 Refs)

35. AVIAN SARCOMA VIRUS UR2 ENCODES A TRANSFORMING PROTEIN WHICH IS ASSOCIATED WITH A UNIQUE PROTEIN KINASE ACTIVITY.

Feldman RA, Wang LH, Hanafusa H, Balduzzi PC
Rockefeller Univ., New York, NY, 10021
J Virol; 42(1):228-236 1982

UR2 is a newly characterized avian sarcoma virus whose genome contains a unique sequence that is not related to the sequences of other avian sarcoma virus transforming genes thus far identified. This unique sequence, termed ros, is fused to part of the viral gag gene. The product of the fused gag-ros gene of UR2 is a protein of 68,000 daltons (P68) immunoprecipitable by antiserum against viral gag proteins. In vitro translation of viral RNA and in vivo pulse-chase experiments showed that P68 is not synthesized as a larger precursor and that it is the only protein product encoded in the UR2 genome, suggesting that it is involved in cell transformation by UR2. In vivo, P68 was phosphorylated at both serine and tyrosine residues. Immunoprecipitates of P68 with anti-gag antisera had a cyclic nucleotide-independent protein kinase activity that phosphorylated P68, rabbit immunoglobulin G in the immune complex, and alpha-casein. The phosphorylation by P68 was specific to tyrosine of the substrate proteins. P68 was phosphorylated in vitro at only one tyrosine site, and the tryptic phosphopeptide of in vitro-labeled P68 was different from those of Fujinami sarcoma virus P140 and avian sarcoma virus Y73-P90. A comparison of the protein kinases encoded by UR2, Rous sarcoma virus, Fujinami sarcoma virus, and avian sarcoma virus Y73 revealed that UR2-P68 protein kinase is distinct from the protein kinases encoded by those viruses by several criteria. Our results suggest that several different protein kinases encoded by viral transforming genes have the same functional specificity and cause essentially the same cellular alterations. (Author abstract) (32 Refs)

36. ACTIVATION OF CYCLIC AMP-DEPENDENT PROTEIN KINASE BY EPINEPHRINE IN PROLIFERATING LYMPHOID CELLS.

de la Houssaye BA, Michnoff CA, Masaracchia RA
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Arch Biochem Biophys; 214(2):610-621 1982

Specific methods for analyses of protein kinase activation which minimize dissociation and/or reassociation of the subunits during extraction and assay were used to analyze cyclic AMP-dependent protein kinase activity in intact lymphosarcoma cells. Such methods did not accurately reflect the activity of the enzyme in hormone-treated cells. Methods involving a two-buffer extraction procedure were devised as the optimum protocol for determining in vivo activation of Isozyme I cyclic AMP-dependent protein kinase. (38 Refs)

37. CANDIDATE PRODUCT OF THE FBJ MURINE OSTEOSARCOMA VIRUS ONCOGENE: CHARACTERIZATION OF A 55,000-DALTON PHOSPHOPROTEIN.

Curran T, Teich NM
Imperial Cancer Res. Fund Lab., Lincoln's Inn Fields, London WC2A 3PX, England
J Virol; 42(1):114-122 1982

Sera from rats bearing tumors induced by inoculation of FBJ murine osteogenic sarcoma virus (FBJ-MSV) nonproducer rat cells precipitate two proteins with mol wt of 55,000 (p55) and 39,000 (p39) from FBJ-MSV-transformed cells. These proteins cannot be precipitated from uninfected cells or cells transformed by other strains of murine sarcoma virus, nor can they be precipitated by sera specific for the viral structural proteins. A methionine tryptic peptide mapping analysis showed that p55 and p39 have little or no homology and that they are not related to the helper virus gag and env gene products. p55 could also be detected among the in vitro translation products of 70S RNA from FBJ murine leukemia virus plus FJB-MSV virions but not among those from FBJ murine leukemia virus alone. This suggests that p55 is encoded by the FBJ-MSV genome, whereas p39, which was not detected among the in vitro translation products, may not be virus encoded. Another difference between p55 and p39 is that p55 is phosphorylated, with most of the phosphate on a serine residue(s), whereas p39 is phosphorylated to a much lesser extent, if at all. No protein kinase activity was associated with p55 and p39 immune complexes under standard conditions. Our data suggest that p55 is a strong candidate for the FBJ-MSV oncogene product. (Author abstract) (32 Refs)

38. RETINOIC ACID INDUCES ANCHORAGE- AND DENSITY-DEPENDENT GROWTH WITHOUT RESTORING NORMAL CYTOSKELETON, EGF BINDING, FIBRONECTIN CONTENT AND ODC ACTIVITY IN A RETROVIRUS-TRANSFORMED MOUSE CELL LINE.

Mukherjee BB, Mobry PM, Pena SD
Dept. Biology, Center Human Genetics, Montreal, Quebec H3A 1B1, Canada
Exp Cell Res; 138(1):95-107 1982

We studied the effects of retinoic acid (RA), a known tumor inhibitor, on a group of transformation-related properties expressed by a Kirsten sarcoma virus (Ki-MSV)-transformed BALB/c 3T3 (clone A31) cell line (KA31). The aim was to determine whether any one or a group of such transformation-specific changes, exhibited by this cell line, is an absolute prerequisite for its acquisition of the neoplastic state. Our study shows that RA treatment restores two important parameters of normalcy in KA31 cells: density-dependent growth inhibition, and anchorage-dependent growth. Retinoic acid also induces cell-to-substratum adhesiveness in a significant proportion of the treated KA31 cell population. However, four other markers of transformation - loss of microfilaments, lack of surface fibronectin, reduced epidermal growth factor (EGF) binding, and increased ornithine decarboxylase activity - remain unaltered by RA treatment. These transformation-specific changes, therefore, are dissociable from loss of anchorage- and density-dependent growth. It is suggested that RA may intervene in transforming protein kinase-induced cell surface changes that are involved in cellular malignancy and the loss of growth control in KA31 cells. (Author abstract) (57 Refs)

39. HALF-LIFE OF THE ROUS SARCOMA VIRUS TRANSFORMING PROTEIN PP66SRC AND ITS ASSOCIATED KINASE ACTIVITY.

Ziemiecki A, Friis RR, Bauer H
Institut fur Virologie, Fachbereich Humanmedizin, Justus-Liebig-Universitat, 6300 Giessen, W. Germany
Mol Cell Biol; 2(4):355-360 1982

Chick embryo cells infected with the Prague A strain of Rous sarcoma virus and several transformation-defective, temperature-sensitive mutants were pulse-labeled with [35S]methionine; the half-lives of metabolically labeled pp60src and the associated protein kinase activity were determined. Irrespective of the temperature at which the cells were grown, pp60src had a half-life of approx 60 min in Wild-type and mutant-infected cells. Treatment with cycloheximide, emetine, cordycepin, or puromycin significantly prolonged the half-life of the pp60src-associated protein kinase activity and the half-life of metabolically labeled pp60src. The apparent contradiction between the half-lives of the kinase activity and the labeled pp60src protein was resolved by the observation that treatment of cells with inhibitors of protein synthesis stabilized pp60src, resulting in a greatly extended half-life. Since protein synthesis inhibitors also extended the half-life of the gag precursor polypeptide, Pr76, a host factor(s) may be necessary for efficient intracellular processing of the polypeptide to the gag proteins. (30 Refs)

40. CONSTRUCTION OF PLASMIDS FOR EXPRESSION OF ROUS SARCOMA VIRUS TRANSFORMING PROTEIN, P60SRC, IN ESCHERICHIA COLI.

Gilmer TM, Parsons JT, Erikson RL
Dept. Pathology, Univ. Colorado Sch. Medicine, Denver, CO, 80262
Proc Natl Acad Sci USA; 79(7):2152-2156 1982

We have constructed plasmids that direct the synthesis of the Rous sarcoma virus transforming gene (src) product (p60src) in Escherichia coli. A 203-base-pair lac promoter-operator DNA encoding the first eight amino acids or beta-galactosidase was ligated to the 5' end of the src gene from the Prague A strain of Rous sarcoma virus (PrA-RSV) which had been cloned in pBR325. Antiserum, from a tumor-bearing rabbit, directed against pp60src was used to screen bacteria containing the recombinant plasmid for a protein of approx 60,000 daltons, and several colonies producing a protein immunologically related to pp60src were detected. Partial proteolytic cleavage analysis revealed that the src-related protein produced in bacteria is structurally similar to pp60src immunoprecipitated from PrA-RSV-infected chicken cells. Partially purified src protein from E coli can be phosphorylated in vitro by the catalytic subunit of cAMP-dependent protein kinase. Tryptic phosphopeptide analysis demonstrated that the catalytic subunit phosphorylated a serine-containing tryptic peptide in the bacterial src protein that comigrated with the phosphoserine-containing tryptic peptide of pp60src immunoprecipitated from 32P-labeled PrA-RSV-infected chicken cells. (Author abstract) (31 Refs)

OTHER RELATED STUDIES

41. ANTIBODIES AGAINST TSH RECEPTOR IN GRAVES' DISEASE: EVALUATION BY RELEASE OF CAMP.

Gomez JM, Navarro MA, Pastor MC, Soler J

Endocrinology Section, Cuidad Sanitaria 'Principes de Espana', Barcelona, Spain

Protides Biol Fluid Proc Colloq; 29:571-574 1982

Release of cyclic AMP (cAMP) was used to evaluate antibodies against thyroid-stimulating hormone (TSH) receptor in 35 patients with active Graves' disease (GD: with or without previous antithyroid treatment), 27 with treated GD, 12 in remission of GD, 12 with multinodular goiter, 10 with primary hypothyroidism, 6 with thyroid cancer, and 84 controls. TSH antibodies were identified in at least 92% of patients with hyperthyroidism of GD and patients with active GD released a significantly greater amount of cAMP than controls and patients with GD in remission. (8 Refs)

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AUTHOR INDEX WITH CITATION NUMBERS

Agus ZS 29
Alitalo K 16
Amano T 30
Aultman KS 20
Azzara A 1
Balduzzi PC 35
Barouski-Miller 12
Bauer H 39
Bloom BR 21
Bloom BR 9
Carlson J 28
Cassel D 33
Cooke BA 25
Costlow ME 13
Cramer H 6
Criss WE 26
Cuesta Casado M 19
Curran T 37
Darman PS 27
De la Houssaye 36
Dix CJ 25
Elgebaly S 3
Erikson RL 40
Feldman RA 35
Forgue-Lafitte 24
Forsberg JG 4
Frazier RP 13
Friis RR 39
Gelehrter TD 12
Gilmer TM 40
Glaser L 33
Goldfarb S 29
Gomez JM 41
Gonda MA 17
Hadley ME 27
Hanafusa H 35
Higashida H 23
Hruby VJ 27
International C 8
Ishizaka T 21
Jarett L 22
Johnson LF 18
Kang YH 26
Kano-Tanaka K 23
Katada T 30
Kato K 23
Kempf J 34
Kiechle FL 22
Koshcmeenko NN 32
Kunkel S 3
Kurkinen M 16
Kvinnsland S 4
Laburthe M 24
Larner EH 31
Lonning PE 4
Lovett EJ 3
Mayes JP 20
Masaracchia RA 36
Mellstrom K 16
Michnoff CA 36
Miki N 23
Mobry PM 38
Mukherjee BB 38
Muramatsu H 15
Muramatsu T 15
Nagatsu T 23
Nakano T 23
Navarro MA 41
Notario A 1
Parker JC 22
Parsons JT 40
Pastor MC 41
Pena SD 38
Perversi L 1
Petrucci E 1
Quan PC 21
Quirin-Stricker 34
Rager-Zisman B 9
Ramos EH 19
Rapp UR 17
Riazanov EM 5
Richelson E 28
Rizzino A 17
Rosen OM 9
Rosselin G 24
Rubin H 2
Rutherford CL 31
Sahai A 26
Sawyer TK 27
Schindler E 6
Schmitt M 38
Schneck J 9
Schumacher M 25
Smith A 28
Snoek GT 10
Soler J 41
Stellwagen R 11
Strinden ST 11
Suzuki F 14
Takano T 14
Takigawa M 14
Tanaka T 23
Teich NM 37
Ui M 30
Vaheri A 16
Van Wijk R 10
Varani J 3
Virtanen I 16
Voorma HO 10
Wang LH 35
Wasserstein A 29
West WL 26
Wu JS 18
Xu B 7
Ziemiecki A 39
Zocchi MT 1
Zweibaum A 24

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SEPTEMBER, 1982

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In this issue:

- **REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES** **1-8**
- **REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES** **9-10**
- **CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES** **11-17**
- **CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS** **18-27**
- **CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES** **28-30**
- **PROTEIN KINASE STUDIES** **31-44**
- **OTHER RELATED STUDIES** **45-47**

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HE 20.3173/2:

REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES

1. BIOCHEMICAL CHANGES IN EXPERIMENTAL RAT BRAIN TUMORS INDUCED BY ETHYLNITROSOUREA AND HUMAN GLIOMAS AFTER DIBUTYRYL CYCLIC AMP TREATMENT.

Shibata I, Matsumoto M, Nagasawa S, Seiki Y, Takezawa J
Dept. Neurosurgery, Toho Univ., Sch. Medicine, 6-11-1, Omori-nishi, Ota-ku, Tokyo 143, Japan
Neurol Med Chir (Tokyo); 22(1):1-6 1982

Previous reports have shown that dibutyryl 3',5'adenosine monophosphate (DBc-AMP) suppresses growth of actively dividing tumor cells, accelerates normalization of cellular metabolism and elevates the intracellular level of cyclic AMP. In an effort to study this mechanism further and define its significance, studies were performed on pregnant rats with ethylnitrosourea (ENU) induced gliomas and human subjects with malignant gliomas. RNA and DNA were detected in remarkably high concentrations in the tumor tissue with a significant reduction after treatment with DBc-AMP. The reduction was more pronounced with RNA. Free amino acids were noted in relatively low concentrations in tumor tissue. However, treatment with DBc-AMP resulted in a significant elevation of their value. The levels of glutamic acid, aspartic acid and GABA increased to levels corresponding to the composition of free amino acids in normal brain tissues. Lysosomal enzyme activity in tumor tissue was 10x greater than that in normal brain tissue. This activity dropped significantly after administration of DBc-AMP. In rats with ENU induced glioma, the uptake of radioactive [H3]DBc-AMP by tumor tissue occurred in a volume 2-3x that of normal tissue. However, the ratio of [H3]cyclic AMP/cyclic AMP was less than that in normal tissue, implying that an enhanced synthesis of endogenous cyclic AMP occurs after administration of DBc-AMP. (Author abstract) (22 Refs)

2. CELL GROWTH AND TRNALYS4 SYNTHESIS IN MOUSE 3T3 CELLS.

Ortwerth BJ, Wolters J, Nahlik J, Conlon-Hollingshead C
Dept. Ophthalmology, Univ. Missouri, Columbia, MO, 65201
Exp Cell Res; 138(2):241-250 1982

The RPC-5 chromatographic profiles of lys-tRNA were analyzed during the growth of 3T3 cells in culture. An inverse relationship was seen between tRNAlys2 and tRNAlys4 which was markedly influenced by medium changes. This interchange of tRNAlys2 and tRNAlys4 could be controlled by altering the levels of serum in the medium, or more precisely by altering the serum to cell ratio. A different change in lys-tRNA distribution was seen when the cells reached confluency. The amounts of tRNAlys2, tRNAlys3 and tRNAlys4 all decreased with a corresponding increase in either tRNAlys5 or tRNAlys6. An identical change in lys-tRNA could be produced by shifting sparse cells into a medium containing 10% calf plasma instead of 10% serum. Both tRNAlys profiles and cell growth were returned to normal when the cells were returned to medium with 10% fetal calf serum (FCS) or 10% calf plasma and fibroblast growth factor (FGF). A third alteration in tRNAlys profiles was seen by the addition of cAMP to the cultures. A decrease in tRNAlys5 and a corresponding increase in tRNAlys6 was seen upon the addition of 10(-3) M db-cAMP and was accentuated by the simultaneous addition of 10(-3) M methyl isobutylxanthine. These data are consistent with an ordered sequence of tRNAlys modification involving tRNAlys2, tRNAlys3, tRNAlys4, tRNAlys5B and tRNAlys6. Several of the factors which control proliferation appear to control the activity of different tRNA-modifying enzymes in this tRNAlys pathway thereby controlling the levels of tRNAlys4, a tRNA previously shown to correlate directly with the proliferative rate of cells. (Author abstract) (23 Refs)

3. INHIBITION OF MOUSE NEUROBLASTOMA CELLS BY PROTEIN FACTOR DERIVED FROM RAT GLIOBLASTS.

Kato T, Sakazaki Y, Yamakawa Y, Kato H, Naganawa N, Kato T, Tsunooka H, Masaoka A, Tanaka R
Dept. Biochemistry (1st Div.), Nagoya City Univ. Medical Sch., Mizuho-ku, Nagoya 467, Japan
Dev Brain Res; 3(4):645-651 1982

A neuroblastoma growth-inhibition factor (NGIF), isolated from the medium of cultured fetal rat glioblasts, inhibited growth and DNA synthesis in cultured mouse neuroblastoma cells (Neuro-2a, NS-20Y, or N1E-115). NGIF promoted the morphologic differentiation of the mouse neuroblastoma cells but did not reduce their viability. NGIF did not increase intracellular cyclic AMP or cyclic guanosine monophosphate levels in the mouse neuroblastoma cells. At concentrations which significantly inhibited DNA synthesis in the mouse neuroblastoma cells, NGIF enhanced DNA synthesis in rat C6 glioma cells and had no effect in mouse neuroblastoma-like cells (Swiss 3T3 or 3T6) or rat glial cell lines LRM-55 and 354A. Exposure to NGIF did not affect the proliferation or morphologic differentiation of the glial cell lines, suggesting that under physiologic conditions it may act specifically on neuronal cells. High concentrations (greater than 25%) of fetal bovine serum in the medium abolished the inhibitory effects of NGIF on DNA synthesis in mouse neuroblastoma cells, suggesting that NGIF may be inactivated by serum soon after its migration to the bloodstream. The apparent mol wt (75,000), isoelectric point (5.8), absence of esterase activity, and biologic activities of NGIF differed from those of glial factor (isolated from C6 rat glioma) and gamma subunit of nerve growth factor. A/J mice inoculated with NGIF-exposed neuroblastoma cells survived longer than controls inoculated with untreated cells, although the times to palpable tumor appearance were about the same in the two groups. Daily injection of NGIF into the region adjacent to the tumor reduced tumor growth and significantly prolonged the survival time. (11 Refs)

4. ROLE OF NATURAL PROSTAGLANDINS IN THE CONTROL OF MURINE MAMMARY TUMOR VIRUS EXPRESSION.

Svec J, Svec P, Halcak L, Thurzo V
Cancer Res. Inst., Slovak Acad. Sciences, Csl. armady 21, CSSR-880 32 Bratislava, Czechoslovakia
J Cancer Res Clin Oncol; 103(1):55-67 1982

The regulation of murine mammary tumor virus (MuMTV) production by mammotropic hormones, hormonomimetic substances, and cyclic nucleotides was investigated. The virus produced in control and treated mammary tumor cell cultures was quantitated by measuring the supernatant reverse transcriptase activity in exogenous reaction using poly(rC).oligo(dG) as template-primer. Two days after exposure, the synthetic glucocorticoid, dexamethasone (DXMT), increased spontaneous MuMTV production at optimal concentration (0.1 umol) up to 10x. Dibutyryl derivative of cyclic AMP had no effect on spontaneous MuMTV production, whereas the drug potentiated suboptimal concentrations of the glucocorticoid. Natural prostaglandins, potent agonists of adenylate cyclase catalyzing intracellular synthesis of cyclic AMP, enhanced both basal (up to 5x) and DXMT-stimulated (up to 1.6 times) MuMTV replication. The MuMTV-stimulating activity of prostaglandins decreased in the order of PGA1 greater than PGE1 greater than PGB1 greater than PGF2 alpha. Prostaglandins can be replaced partially by norepinephrine and isoproterenol by enhancing the DXMT-mediated MuMTV stimulation, whereas these drugs remained without effect on spontaneous MuMTV production. Theophylline, an antagonist of cAMP-phosphodiesterase converting cAMP to AMP, enhanced the virus-stimulating activity of DXMT as well as of prostaglandins. The enhancement of MuMTV production by adenylate cyclase agonists do not correlate absolutely with the estimates of intracellular cAMP levels, since the highest amounts of cAMP have been repeatedly observed in cells treated with PGE1 and norepinephrine. The results indicate that besides hormones, other hormone-like substances and cyclic nucleotides may be involved in the complex mechanism of hormone-regulated MuMTV genome expression. (Author abstract) (55 Refs)

5. CHANGES OF ORNITHINE DECARBOXYLASE ACTIVITY AND POLYAMINE CONTENT UPON DIFFERENTIATION OF MOUSE NB-15 NEUROBLASTOMA CELLS.

Chen KY, Presepe V, Parken N, Liu AY
Dept. Chemistry, Rutgers-The State Univ. New Jersey, New Brunswick, NJ, 08903
J Cell Physiol; 110(3):285-290 1982

Specific changes of basal level of ornithine decarboxylase (ODC) activity and spermidine content during the differentiation of murine NB-15 neuroblastoma cells and the temporal relationship of these changes and the expression of differentiated phenotypes of neuroblastoma cells are reported. ODC activity of nondifferentiating (NB) neuroblastoma cells and differentiating (ND) cells was studied over a 5 day period. NB cells showed an early increase in ODC activity that was temporarily associated with the resumption of logarithmic growth after plating. Upon entering the stationary phase of cell growth, ODC activity in NB cells decreased precipitously, reaching a minimum activity of 0.2 units/mg protein at the end of the 5 day culture period. In ND cells, the early increase in ODC activity was attenuated in both magnitude and duration. The increase in ODC activity of ND cells was transient. While putrescine levels were similar in NB and ND cells. Significant differences were observed in levels of cellular spermidine. Spermidine content of NB cells followed a steady increase from the basal level of 1.5 nanoMols (nM)/mg protein to a maximal value of 5.05 nM/mg protein. This elevated level of spermidine in NB cells was sustained for the entire 5 day period. The significance of cAMP-induced changes in ODC activity and polyamine contents in cell differentiation are discussed. (15 Refs)

6. ROUS SARCOMA VIRUS TRANSFORMED CELLS ARE RESISTANT TO CYCLIC AMP.

Roth CW, Singh T, Pastan I, Gottesman NM
Lab. Molecular Biology, NCI, Building 37, Room 2E24, Bethesda, MD, 20205
J Cell Physiol; 111(1):42-48 1982

Rous sarcoma virus (RSV)-transformed Chinese hamster ovary (CHO) cells were found to differ from spontaneously transformed CHO cells in their sensitivity to growth inhibition or changes in cell shape induced by 8-Br-cyclic AMP (BrcAMP) treatment. RSV-transformed rat (NRK) cells were also observed to have reduced sensitivity to BrcAMP. These results indicate that transformation of CHO and NRK cells by RSV alters the cells in a different manner than spontaneous transformation. (31 Refs)

7. SECRETION OF CALCITONIN AND PARATHYROID HORMONE BY EMBRYONAL CARCINOMA CELLS: VARIATIONS WITH DIFFERENTIATION (MEETING ABSTRACT)

Evain D, Binet E
Inserm U 188 Av, Denfert Rochereau, 75014 Paris, France
Pediatr Res; 15(12):1552 1981

Differentiation of teratocarcinoma cells in culture allows us to study certain aspects of the early embryogenesis. The adenylate cyclase system of cultured F9 cells is specifically stimulated by calcitonin. Differentiation into endodermal cells by treatment with retinoic acid is followed by a loss of calcitonin responsiveness and the appearance of parathyroid hormone stimulation of the cyclase system. In addition radioimmunological and biological active forms of calcitonin are secreted by F9 cells in the culture medium. Along with differentiation with retinoic acid a progressive decrease of calcitonin secretion is observed: after 4 days of treatment calcitonin is no longer detectable. But at this stage of differentiation a parathyroid hormone-like substance is radioimmunologically and biologically detectable. These specific changes in hormonal secretion and receptivity can be used as markers of differentiation. These results also suggest a possible role for Calcitonin and Parathyroid hormone-like activities during the initial steps of embryogenesis. (no Refs)

8. DIBUTYRYL CAMP AND NERVE GROWTH FACTOR INDUCED DIFFERENTIATION OF HUMAN NEUROBLASTOMA (MEETING ABSTRACT)

Prashad N
Graduate School of Biomedical Sciences, University of Texas Houston, State College Houston, TX, 77025
Med Pediatr Oncol; 9(5):526 1981

Human neuroblastoma tumors spontaneously differentiate into ganglioneuroma in vivo, causing them to regress spontaneously. We are investigating the mechanisms involved in differentiation by studying the effects of dibutyryl cAMP and nerve growth factor (NGF) of cAMP binding proteins (cAMP BPs) and protein kinases (PKs) in human neuroblastoma cell lines. One mM dibutyryl cAMP and NGF (250ng/ml) increased cAMP BPs by 2.5 and 1.5-fold. dibutyryl cAMP activiated cAMP dep-PKs in vivo, in contrast, NGF did not activate PKs. cAMP were identified by the photoactivation of cytoplasmic proteins plus 8-N(3)-32(P) cAMP and their analysis on 1-di and 2-di gels. 1-di-gels show 3 major cAMP BPs: 54,000 MW (R11) regulatory subunit of PK11, 51,000 MW ('R11) a new cAMP BP and 48,000 MW (R1) regulatory subunit of PKI. Dibutyryl cAMP increased R1 by 3-fold and NGF increased 'R11. 2-di-gels show 4 major cAMP BPs: R11 (54,000 MW, pI4.6), 'R11 (51,000 MW, pI5.0), R1 (48,000 MW, pI5.3) and R (48,000 MW, pI5.4) and 3 minor 'brain-specific' cAMP BPs number 2 to 4 (55,000 MW pI5.6-6.0). Dibutyryl cAMP increased R, induced a new protein 'R (48,000 MW, pI5.5) and BPs number 1 to 4. NGF increased BPs number 2-4. R1, R and 'R seem to be multiple forms of regulatory subunit of PKI and R11 and 'R11 of regulatory subunit of PKII. Whether all the multiple forms of cAMP BPs are associated with catalytic subunit of PKs is not known. Dibutyryl cAMP or NGF induces differentiation of neuroblastoma and induced cAMP BPs may play a role in cellular differentiation. (no Refs)

REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES

9. CELL-TYPE DIFFERENCES IN THE SERUM AND CYCLIC AMP-DEPENDENT REGULATION OF ORNITHINE DECARBOXYLASE ACTIVITY IN TWO CULTURED FIBROBLAST TYPES.

Boucek RJ, Weiss J, Lembach KJ

Dept. Pediatrics and Biochemistry, Vanderbilt Univ. Sch. Medicine, Nashville, TN, 37232

Biochem J; 204(1):357-360 1982

Serum re-feeding stimulated ornithine decarboxylase (ODC) activity 8 to 10-fold in FS fibroblasts and 5 to 8-fold in 3T3 fibroblasts. Addition of dibutyryl cyclic AMP or 3-isobutyl-1-methylxanthine at the time of serum re-feeding further stimulated ODC activity in 3T3 fibroblasts but inhibited the serum stimulation of ODC activity in FS fibroblasts. It is suggested that serum and cyclic AMP independently regulate ODC activity in cultured fibroblasts. (Author abstract) (19 Refs)

10. INHIBITION OF ORNITHINE DECARBOXYLASE AND S-ADENOSYLMETHIONINE DECARBOXYLASE ACTIVITIES OF S49 LYMPHOMA CELLS BY AGENTS INCREASING CYCLIC AMP.

Honeysett JM, Insel PA

Div. Pharmacology, M-013H, Dept. Medicine, Univ. California, San Diego, La Jolla, CA, 92093

J Cycl Nucleotide Res; 7(5):321-332 1981

Regulation of two key enzymes which control polyamine biosynthesis, L-ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (SAMDC), by agents increasing cyclic AMP (cAMP) was studied in various S49 lymphoma cell lines. Incubation of wild type S49 cells with beta-adrenergic agonists (terbutaline or isoproterenol) led to more rapid inhibition (less than 2 hr) of activities of both enzymes than cells in the G1 phase. Propanolol (beta-adrenergic antagonist) blocked inhibition of ODC by isoproterenol but only if added simultaneously or less than 4 hr after the agonist. Decreases in ODC activity produced by the beta-adrenergic agonists, cholera toxin, prostaglandin E1 or dibutyryl cAMP were all enhanced by the phosphodiesterase inhibitor Ra 20-1724. Results of studies of ODC and SAMDC activity in S49 variants having lesions in the pathway of cAMP generation and action indicate that the 'classical' components of the hormone receptor-cAMP-protein kinase pathway are involved. These results in S49 cells contrast with evidence in other systems in which cAMP has been suggested to enhance polyamine biosynthesis, perhaps through alternative mechanisms. (36 Refs)

CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES

11. SOLUBILIZATION OF EPINEPHRINE-SPECIFIC ALPHA2-ADRENERGIC RECEPTORS FROM ADRENOCORTICAL CARCINOMA.

Nambi P, Aiyar NV, Sharma RK
Dept. Biochemistry, Univ. Tennessee, Memphis, TN, 38163
FEBS Lett; 140(1):98-102 1982

Using a zwitterionic detergent, alpha-receptors were selectively solubilized from adrenocortical carcinoma 494. The isolated receptors were determined to be exclusively of the alpha2-subtype. A major function of these receptors may be the mediation of epinephrine-induced transmembrane signal via the activation of guanylate cyclase. The alpha2-adrenergic coupled guanylate cyclase system might be one of the means of transmitting the catecholamine-sensitive biological response in the adrenocortical carcinoma cell. (27 Refs)

12. ENHANCEMENT OF HORMONE ACTION BY A PHORBOL ESTER AND ANTI-TUBULIN ALKALOIDS INVOLVES DIFFERENT MECHANISMS.

Simantov R, Sachs L
Dept. Genetics, Weizmann Inst. Science, Rehovot 76100, Israel
Biochim Biophys Acta; 720(2):120-125 1982

The tumor-promoting phorbol ester 12-O-tetradecanoyl phorbol-13-acetate (TPA) enhanced 1-isoproterenol and prostaglandin E1 stimulated cyclic AMP formation in clones of mouse myeloid leukemic cells. The enhancement was found up to 3 hr after TPA treatment and had disappeared after 24 hr, indicating its reversibility. The effect of TPA was not inhibited by removal of extracellular Ca++ or pretreatment with the calcium ionophore A23187. This enhancement by TPA seems to involve a different pathway than enhancement of response to the same hormones after treatment with the anti-tubulin alkaloids colchicine or vinblastine, since a myeloid leukemic cell mutant clone that was non-responsive to the anti-tubulin alkaloids responded to TPA. Furthermore, combined treatment of colchicine-sensitive cells with TPA and colchicine showed an additive stimulating effect. The enhancement of cell response to hormones by TPA was found in myeloid leukemic cell clones that either were or were not induced to differentiate after treatment with TPA. This suggests that enhancement of the effect of these and possibly other hormones by TPA may be an initial step of TPA action, but that this enhancement is not sufficient to induce the wide repertoire of TPA effects including induction of differentiation. (Author abstract) (36 Refs)

13. DESENSITIZATION OF PGE1 RECEPTORS IN NEUROBLASTOMA-GLIOMA HYBRID CELLS.

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Prostaglandins; 23(3):311-318 1982

Prostaglandin E1 receptor sites were measured in homogenates of NG108-15 neuroblastoma-glioma hybrid cells after exposure of intact cells to PGE1. Scatchard analysis of competitive binding studies showed that incubation of NG108-15 cells in the presence of 2.5 uM PGE1 for 16 hr resulted in a loss of PGE1 receptors and an increase in the dissociation constant of the remaining receptors. Thus, cells challenged with

PGE1 not only lose adenylate cyclase activity, but also lose PGE1 receptors and decreased the affinity of the remaining receptors for PGE1. (Author abstract) (15 Refs)

14. EPINEPHRINE-INDUCED CHANGES IN THE CYCLIC NUCLEOTIDE CONTENT OF FISH MELANOMA CELLS.

Negishi S, Masada M, Wakamatsu Y, Ohoka T, Obika M
Dept. Biology, Keio Univ., Yokohama 223, Japan
Gen Comp Endocrinol; 47(1):88-93 1982

Intracellular levels of adenosine 3',5'-monophosphate (cyclic AMP) and guanosine 3',5'-monophosphate (cyclic GMP) in melanoma tissues developed in platyfish and swordtail hybrids were determined by radioimmunoassay. The melanoma tissues were composed mostly of pigment cells, many of which responded to epinephrine with pigment aggregation. The aggregated melanosomes redispersed gradually by washing the cells with physiological saline, and rapidly by the perfusion with theophylline. Determination of intracellular levels of cyclic nucleotides revealed that epinephrine produced a small but significant decrease in cyclic AMP level, whereas theophylline elicited a remarkable increase in the intracellular concentration of cyclic AMP. On the other hand, the change in the levels of cyclic GMP was not significant among the tissues subjected to the different experimental conditions. The present results suggest that the centrifugal and centripetal translocations of melanosomes within melanophores are regulated directly by the intracellular concentration of cyclic AMP. (Author abstract) (37 Refs)

15. PROPERTIES AND CHARACTERISTICS OF ADENYLATE CYCLASE IN NORMAL AND FIBROCYSTIC BREAST EPITHELIAL CELLS IN HUMANS (MEETING ABSTRACT)

Foecking MK, Abou-Issa H, Minton JP
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Fed Proc; 41(3):1409 1982

Basal, fluoride and hormone-stimulated activities of adenylate cyclase (AC) have been studied in mammary epithelial cells of human tissues freed from adipose tissue. Epithelial cells were isolated by collagenase treatment of normal and fibrocystic breast tissues. The apparent Km for MgATP2-in both tissues is 1.0x10(-4) M. AC in both tissues is stimulated only by catecholamines and prostaglandins (PGE1, PGE2). The enzyme was maximally activated (2 x basal activity) by L-epinephrine (EPI) with an apparent Km of 2.0 x 10(-6) M. Norepinephrine also stimulated the activity but with an increased apparent Km of 8 x 10(-5) M. PGE1 and PGE2 (at 10(-6) M) stimulated the basal activity of AC by 140% and 95% respectively. EPI and PGE1 together have no additive stimulatory effect indicating separate receptor sites for each hormone. The stimulation by EPI or PGE1 is potentiated 4-and 2.5-fold respectively by the addition of 10 x 10(-5) M Gpp (NH)p. The apparent Km for Gpp(NH)p is approximately 15 uM. The catecholamine stimulation appears to be mediated through Beta 2 adreno-receptors. AC from fibrocystic breast has an increased responsiveness to EPI compared to normal tissue both in the absence and presence of Gpp(NH)p. However no difference was observed in PGE1-responsiveness in both tissues. The results suggest increased catecholamine responsiveness play a developmental role in fibrocystic breast disease. (no Refs)

16. CYCLIC NUCLEOTIDES IN MONONCLEAR CELLS ISOLATED FROM BONE MARROW OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) (MEETING ABSTRACT)

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Fed Proc; 41(3):1413 1982

As a part of our continuing studies on the participation by cyclic nucleotide - related systems in the proliferative behavior of leukemic cells we have assayed the intracellular concentrations of both adenosine 3':5' monophosphate (cAMP) and guanosine 3':5' monophosphate (cGMP) in isolated mononuclear bone marrow cells during short - term incubations. Additions of sodium nitroprusside resulted in large increases of cGMP concentration and in much smaller increases of cAMP; 1-methyl-3-isobutylxanthine increased equally concentrations of both nucleotides. Both isoproterenol and norepinephrine increased concentrations of cAMP in cells isolated from the patients undergoing a remission of ALL; serotonin showed a stimulatory effect on the guanylate cyclase activity. Hydrocortisone depressed levels of both cyclic nucleotides. The basal levels of cyclic nuceotides were different in cells isolated from patients at diagnosis and those isolated 2-4 weeks after initiation of therapy. Moreover the response to hormones and drugs changed significantly during the remission. Parallel longer - term suspension cultures indicate that there may be a correlation between the intracellular concentrations of cyclic nucleotides and the cell proliferation rates which could be useful in evaluation of ALL patients. (no Refs)

17. INHIBITION OF DIMETHYLBENZANTHRACENE-INDUCED TUMORS IN RATS BY STRESS (MEETING ABSTRACT)

Goldman PR, Vogel WH
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Fed Proc; 41(3):1559 1982

Previous reports have indicated that various types of physical and psychological stressors inhibit the growth of 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumors in Sprague-Dawley (S-D) rats. Dopamine (DA)-stimulated adenylate cyclase activity in the caudate nucleus has been claimed to be inversely associated with spontaneous mammary tumors in mice. We tried to replicate some of these findings and investigate some factors likely to be involved in this inhibition. Female S-D rats were used for induction of tumors by DMBA. Rats subjected to daily sessions of immobilization 5 days a week for 9 weeks showed a significant decrease in tumor weight when compared with non-stressed rats; 2.7g vs 5.4g per rat, N-20. Housing animals individually significantly increased the size of tumor over group-housed animals, 13.9g vs 5.0g per rat, N-20. However, rats bred for high versus low plasma catecholamine levels in response to stress did not differ in the weight of their tumors. No difference was seen in the number of tumors in any of these groups. Behavioral testing of emotionality prior to DMBA exposure showed no correlation to later tumor formation. DA-stimulated adenylate cyclase activity was found to be lower in animals which are more susceptible to tumor induction, 107 vs 142%, N-11. Short term immobilization stress had no effect on this DA stimulated adenylate cyclase activity. (no Refs)

CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS

18. MODIFICATION OF NEUROBLASTOMA X GLIOMA HYBRID NG108-15 ADENYLATE CYCLASE BY VANADIUM IONS.

Lichtstein D, Mullikin-Kilpatrick D, Blume AJ
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Biochem Biophys Res Commun; 105(3):1157-1165 1982

The effects of various vanadium ions on the adenylate cyclase of mouse neuroblastoma X rat glioma cells which have been well characterized with respect to receptor-mediated activations and inhibitions are reported. Ortho- and metavanadate enhanced enzyme activity at 0.01-1 millimolar (mM); at concentrations greater than 5 nM they inhibited activity. Vanadyl sulfate (0.01-01 mM) increased enzyme activity; although 0.1 mM also inhibited activity. Receptor mediated inhibitory processes appeared to be independent of the level of catalytic activity. (29 Refs)

19. DECREASED ACCUMULATION OF CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE IN 'ISCHEMIC' SKIN AFTER 12-O-TETRADECANOYL-PHORBOL-13-ACETATE TREATMENT.

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J Invest Dermatol; 78(4):264-266 1982

The effect of 12-O-tetradecanoyl-phorbol-13 acetate (TPA) on cyclic adenosine 3',5'-monophosphate (cyclic AMP) level in adult mouse skin in response to ischemia was examined. The incubation of skin pieces in a buffered salts medium at 37 C resulted in a rapid accumulation of cyclic AMP. In mouse skin pieces max accumulation (about 6x the basal level) occurred after 2 min incubation and was followed by a rapid decline in the cyclic AMP level. This 'ischemic' rise in epidermal cyclic AMP was greatly reduced if skin was used 16 hr after a single application of 17 nmoles of TPA. The effect of TPA on cyclic AMP accumulation in response to ischemia was first observed at 1 hr after TPA treatment and was maximal at 4 hr. The lack of 'ischemic' response in TPA-treated skin was not related to an increase in the activity of cyclic AMP phosphodiesterase after TPA application. In addition, the accumulation of cyclic AMP in skin in response to both ischemia and exposure to isoproterenol, adenosine, histamine, or prostaglandin E2 was not observed in skin treated with the tumor promoter TPA. (Author abstract) (19 Refs)

20. INHIBITION OF LYMPHOPROLIFERATION BY DIPYRIDAMOLE.

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Biochem Pharmacol; 31(7):1381-1386 1982

Dipyridamole (DP, Persantin) was examined for its effects on the proliferation of mitogen-stimulated murine splenocytes and L1210 leukemia cells. In keeping with its reported activity as an inhibitor of nucleoside transport, DP inhibited incorporation by lymphoid cells of labeled thymidine and uridine into macromolecules. That this inhibition resulted from activities in addition to suppression of nucleoside transport was verified by measured decreases of cellular DNA and viable cell numbers. In addition, protein synthesis was also decreased as indicated by labeled valine incorporation and total protein content of the cells. The rapid accumulation of cAMP in phytohemagglutinin-stimulated splenocytes in the presence of DP may provide an explanation for the anti-proliferative effect of DP on lymphoid cells. (Author abstract) (8 Refs)

21. INHIBITON BY ETAZOLATE (SQ 20009) AND CARTAZOLATE (SQ 65396) OF ADENOSINE-STIMULATED [3H]CAMP FORMATION IN [2-3H]ADENINE-PRELABELED VESICLES PREPARED FROM GUINEA PIG CEREBRAL CORTEX.

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Biochem Pharmacol; 31(7):1441-1442 1982

Etazolate (SQ 20009) and cartazolate (SQ 65396) were tested as adenosine antagonists; presumably some phosphodiesterase inhibitors produce some of their pharmacological effects principally by antagonizing adenosine. Preliminary studies on adenylate cyclase of vesicle preparations from guinea pig cerebral cortex stimulated by low concentrations of adenosine, in keeping with adenosine concentration in brain, showed that etazolate and cartazolate are at least as potent as theophylline. Etazolate, cartazolate, theophylline, and caffeine displaced the adenosine concentration-activity curves in a manner consistent with a competitive action at the adenosine receptor. (24 Refs)

22. EXTINCTION OF HORMONE-DEPENDENT MAMMARY CANCER IN VIVO AND IN VITRO BY CHLOREA TOXIN (MEETING ABSTRACT)

Clair T, Shepheard C, Cho-Chung YS
National Cancer Institute, National Institutes of Health, Bethesda, MD, 20205
Proc Am Assoc Cancer Res; 23:912 1982

Growth of 4,12-dimethylbenz(a)anthracene (DMBA) induced mammary carcinoma in rat was arrested by daily SC injections of cholera toxin, the exo-enterotoxin of the bacterium Vibrio Cholerae. At a dose of 2 ug/200 g rat, tumors regressed to 50% of their initial size within 2-3 weeks and 85% of tumors regressed completely within 4-6 weeks. The same response to cholera toxin was observed with other hormone-dependent mammary tumor, MTW9, but not with hormone-inedpendent tumors, DMBA number 1 and MT 13762. The latter result was consistent with the lack of response of these hormone-independent tumors to hormone-removal (ovariectomy) or dibutyryl cyclic AMP treatment. The growth inhibitory effect of cholera toxin was dose-dependent and upon cessation of treatment tumors resumed growth; after complete regression, however, tumors did not reappear until 6 months of after termination of the treatment. The amount of cholera toxin as high as 10 ug/day/ 200 g rat injected over 6 weeks period was not toxic to the animals. The growth of human breast cancer cells (MCF-7), in vitro was also inhibited by a daily supplement of cholera toxin. At a concentration of 100 ng/ml, the cell replication ceased completely within 2 days and 1 ng/ml exhibited 50% growth inhibition by day 2. The growth inhibitions of both in vivo and in vitro were preceded by marked increases in the cellular cyclic AMP content, adenylate cyclase, and type II cyclic AMP-dependent protein kinase activities as well as a decrease of estrogen binding activity. Thus, extinction of mammary cancer can be achieved by cholera toxin, an agent that increases intracellular cyclic AMP. (no Refs)

23. INHIBITION OF TUMOR GROWTH WITH LOW DIETARY PROTEIN AND WITH DIETARY GARLIC EXTRACTS (MEETING ABSTRACT)

Criss WE, Fakunle J, Knight E, Adkins J, Morris HP, Dhillon G

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Fed Proc; 41(3):281 1982

Dietary preparations from fresh garlic bulbs (Allium sativum) inhibited the growth of Morris hepatoma 3924A. Diets containing 5%, 15%, or 25% casein (each +/-5% lypholized garlic extracts) were fed to rats without and with inoculated tumor tissues. The results were as follows: Low dietary protein decreased tumor growth from 60-70%. All diets containing 5% garlic yielded decreased tumor growth from 10-25%. The dietary garlic extract was non-toxic as no changes were observed in levels of serum albumin, albumin/globulin ratio, urinary creatinine, liver or spleen size, or animal growth. Similar types of studies were performed by subcutaneously injecting a garlic extract into rats bearing this tumor. In the latter studies, tumor growth was slowed by 30-50%, but the injection treatment resulted in toxicity. Since these garlic extracts contain a potent inhibitor of guanylate cyclase, the only enzyme known to produce the growth stimulating substance, cGMP, it is possible that the inhibition of tumor growth by garlic feeding may result from the reduction of tumor cGMP levels. (no Refs)

24. INDUCTION OF ADENYLATE CYCLASE COMPONENTS IN HELA CELLS BY SODIUM BUTYRATE (MEETING ABSTRACT)

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Fed Proc; 41(3):1408 1982

Previous studies have demonstrated that HeLa cells have beta adrenergic receptors (R) but respond poorly to beta-agonists. Cells treated overnight with low (0.5-0.8 mM) or high (5 mM) Na butyrate (5Bu) exhibited a 2-3X increase in R. Whereas isoproterenol (ISO) stimulated cAMP production up to 40X in 5Bu-treated cells, response to ISO was only slightly increased by low Bu. Cholera toxin (CT) elevated cAMP levels more in 5Bu-treated than untreated cells and shifted the dose response to ISO over 50X in both. The dose response was not changed by Bu treatment. ISO stimulated adenylate cyclase 7-10X in membranes from 5Bu-treated cells and only 20-60% in those from untreated or low Bu-treated cells. Cyclase also was activated by incubating membranes with A1 subunit of CT plus NAD. With [32P] NAD, CT labeled two proteins of 42 and 48 kdaltons known to be subunits of the regulatory component (N) of adenylate cyclase. 32P incorporaton into these two proteins doubled in 5Bu-treated cells. Finally, using a reconstitution assay to measure N activity, extracts from 5Bu-treated HeLa were 2-3X more effective in restoring cyclase activity that extracts from untreated or low Bu-treated cells. Our results indicate that untreated HeLa contain R and N which appear to be poorly coupled; that low 5Bu-treatment increases R but not N; and that 5Bu-treatment increases both R and N, which may explain partly the enhanced response of HeLa to agonists. (no Refs)

25. THE EFFECTS OF CYCLOPHOSPHAMIDE AND ITS DERIVATIVES ON ADENYLATE CYCLASE ACTIVITY AND PROTEIN SYNTHESIS (MEETING ABSTRACT)

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Br J Cancer; 44(2):306 1981

Nitrogen mustard chemotherapeutic agents are considered to obtain their effect by cross-linking DNA, though other cellular targets have been implicated. We have examined the effects of cyclophosphamide (CP) and its metabolites on the enzymes of cyclic-nucleotide metabolism and protein synthesis. CP, 4-keto-cyclophosphamide (KP) and phosphoramide mustard (PM) (less than or equal to 15 mmol/l) were found to have no effect on basal or stimulated hepatic adenylate cyclase activity. 4-hydroperoxy-cyclophosphamide (HP, 5 mmol/l), was found to inhibit enzyme activity (50%) and completely abolished the effect of glucagon but only partially abolished the stimulatory effect of fluoride. Whereas CP and KP are 'inactive', PM is a potent alkylating agent and is considered as the 'ultimate' alkylating metabolite of CP. These results may be explained by the unique reactivity of HP. Similar specificity has been observed in the sensitivity of cAMP phosphodiesterase and protein kinase for HP. In a separate series of experiments, we have found that protein synthesis is inhibited by low concentrations of CP metabolites. HP (10 mmol/l), inhibited protein synthesis in rabbit reticulocyte lysate by 50%. PM and HN2 showed only a slight effect at this concentration (up to 10% inhibition). (3 Refs)

26. EFFECTS OF CHOLERA TOXIN ON ADENYLATE CYCLASE ACTIVITY AND ACCUMULATION OF CAMP IN HEPATOCYTES AND HEPATOMA CELLS IN VITRO (MEETING ABSTRACT)

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Acta Pharmacol Toxicol [Suppl] (Copenh); 49(3):22 1981

The experiments described here are the first part of an investigation of the relation between cholera toxin-stimulated adenylate cyclase activity and DNA synthesis and cell growth in cultured hepatocytes and hepatoma cells. Cultured MH1C1 hepatoma cells incubated with cholera toxin (0.02-5.0 nM) for 90 minutes showed a dose-dependent increase in cAMP accumulation in the presence of 2 mM theophylline. Cellular cAMP levels increased from 0.5 pmol/mg protein in untreated control cultures to approximately 200 pmol/mg protein in cultures incubated with 5 nM cholera toxin. Cultured hepatocytes also responded to cholera toxin in a dose-dependent manner. cAMP concentrations increased from 5 pmol/mg protein in control cultures to approximately 60 pmol/mg protein in cultures incubated with 10 nM cholera toxin. Increased adenylate cyclase activity in response to cholera toxin was demonstrated in both cultured hepatocytes and hepatoma cells. In both cell types stimulation with cholera toxin was apparent after an incubation time of 30 minutes and was maximal after 150 minutes. The adenylate cyclase activity then levelled off, but remained slightly elevated as compared to control cultures after exposure to cholera toxin for 24 hours. Preliminary results show that there is a dose-dependent relation between cholera toxin concentrations and adenylate cyclase activity in cultured hepatocytes. After pretreatment for 48 hrs with dexamethasone (0.25 uM) cAMP accumulation in cholera toxin-exposed hepatocyte cultures was strongly increased. In the MH1C1 cells dexamethasone only slightly enhanced cAMP accumulation, dexamethasone did not affect cholera toxin stimulated adenylate cyclase activity in hepatocytes. (no Refs)

27. DISSOCIATION BETWEEN GUANYLATE CYCLASE - INHIBITION AND ANTIPAPILLOMA EFFECT OF RETINOIDS (MEETING ABSTRACT)

Rydell EL, Andersson RG, Axelsson KL, Wikberg JE
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Acta Pharmacol Toxicol [Suppl] (Copenh); 49(3):41 1981

Retinoids (derivates of vit A) has in recent years gained much attention for their marked effects as chemotherapeutic and chemopreventive agents in cancer. The cellular events leading to cell growth and transformation is largely unknown, although cGMP (cyclic guanosine monophosphate) has been implicated as a mediator. The cGMP forming enzyme guanylate cyclase (GC) is known to be activated by different nitro-compounds and most markedly by the carcinogen N-methyl-N'-nitro-N-nitroso-guanidine (MNNG). Since GC was found to be strongly inhibited by retinylacetate, we initiated a study upon the effect of different retinoids on GC-activity in order to find out if any correlation between GC-inhibiting activity and anti-cancer effect existed. Experiments were performed on nitroprusside (NP) stimulated GC from rat liver (37,000xg supernatant). NP stimulation was found to be qualitatively the same as MNNG stimulation. The different retinoids were kindly supplied by F Hoffman La Roche and Co Basel Switzerland, and included both analogues with the vit A core left intact, and aromatic retinoids which has been found to possess very potent antipapilloma effect when tested on mouse in vivo. The effect on GC activity of the different retinoids, as obtained in our experiments, were compared to values for antipapilloma effect compiled from literature. This comparison revealed no clear correlation between GC-inhibition and antipapilloma effect. Thus, GC-inhibiting activity was found for some retinoids which were totally inactive in vivo and, on the contrary, some of the highly potent aromatic retinoids showed no GC-inhibiting activity. It was also difficult to find any resemblance in the molecular formula for different retinoids with GC-inhibiting action, which could explain their effect on the enzyme. (no Refs)

CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES

28. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF PURINE AND PYRIMIDINE BASES, RIBONUCLEOSIDES, DEOXYRIBONUCLEOSIDES AND CYCLIC RIBONUCLEOTIDES IN BIOLOGICAL FLUIDS.

de Abreu RA, Van Baal JM, De Bruyn CH, Bakkeren JA, Schretlen ED
Div. Pediatric Oncology, Dept. Pediatrics, St. Radboud Hosp., Catholic Univ. Nijmegen, Nijmegen, The Netherlands
J Chromatogr; 229(1):67-75 1982

A method is presented for the separation and quantitative determination of compounds normally related to purine and pyrimidine metabolism in biological material. The retention behavior of nucleobases, ribonucleosides, deoxyribonucleosides and cyclic ribonucleotides has been systematically investigated by reversed-phase high-performance liquid chromatography using a non-liner gradient. Ultimately a separation of the purine and pyrimidine compounds was achieved in a 35-min run with an av detection limit of 5-10 pmol per injection. Recoveries of standards added to urine, plasma or serum were 96 +-5%. (Author abstract) (26 Refs)

29. ANTI-TUMOR EFFECT AND TERMINAL DIFFERENTIATION INDUCING ACTIVITY OF PHOSPHODIESTERASE INHIBITORS (MEETING ABSTRACT)

Ambrus CM, Huberman E, Horoszewicz J, Leong SS, Biddle W, Montagna R, Gastpar H, Ambrus JL
Roswell Park Memorial Institute, Buffalo, NY, 14263
Proc Am Assoc Cancer Res; 23:894 1982

In previous studies we found that neoplastic human cells produce more cAMP phosphodiesterases than surrounding normal cells. It was thought that inhibitors of these enzymes may have selective anti-tumor effects. In normal and neoplastic human cell lines in culture inhibitors of cAMP phosphodiesterases were tested for anti-tumor effect by cell count and 3H thymidine incorporation. Cell lines studied include ES-1 malignant melanoma, DAUDI and HR-IK Burkitt's lymphoma, 6410 acute myeloid leukemia, MOLT-4F and CCRF-CEM acute lymphoid leukemia, HL-60 promelocytic leukemia, HT-29 colon adenocarcinoma. RT-4 transitional cell bladder carcinoma, LNCap prostatic carcinoma, BG-27 diploid foreskin fibroblasts and NC-37 normal B lymphocytes. The methylxanthine derivative pentoxifylline and the pyrimido-pyrimidine derivative RA-233 exhibited significant anti-tumor effect. This appears to be related to increase in cAMP levels. There was more effect in the lag phase of cell growth than in the logarithmic growth phase. There was more effect on neoplastic cells than on normal cells, particularly in the lower dose range. In certain cell lines RA-233 was significant terminal differentiation inducing effect by morphologic and enzymologic criteria. (1 Ref)

30. BIOCHEMICAL SUBGROUPING OF BREAST DISEASE (MEETING ABSTRACT)

Estes NC, Glover JL, Miller D, Watanabe A
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Proc Am Soc Clin Oncol; 1:C-28 1982

Although lymph node status and hormone receptor assay have been helpful additions, the prediction of biological activity of breast lesions remains a difficult problem. Minton has compared cyclic adenosine monophosphate (cAMP) measurements in breast tissue and has showed a progressive increase in the level of cAMP from normal to fibrocystic disease, with the highest level in breast cancer specimens. In the rat hepatoma system, Weber has shown an excellent correlation between increasing thymidine kinase (Tk) activity and increased tumor growth rate. We have conducted a feasibility study comparing cAMP and Tk as biochemical measurement of tissue proliferation in the human breast system. Tissue samples were obtained from breast biopsy or mastectomy specimens. All samples were immediately frozen and stored in liquid nitrogen until cAMP and Tk assays were done. Tk and cAMP activity was modeled after Weber and Steiner respectively. Tk levels were elevated in intraductal cancers with positive lymph nodes and negative estrogen receptors (3.3) but decreased when lymph nodes were negative and estrogen receptors were positive (1.9). We conclude that Tk shows improved correlation over cAMP in comparing breast lesions. Further studies are encouraged with the hope of improving predictability of biological behavior of breast lessions. (1 Ref)

PROTEIN KINASE STUDIES

31. CAMP-DEPENDENT PHOSPHORYLATION OF PROTEINS IN TISSUES OF IRRADIATED MICE.

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Faculty Biology, M. V. Lomonosov State Univ., Moscow, USSR
Radiobiologiia; 22(1):96-99 1982

The effect of ionizing radiation on cyclic AMP (cAMP)-dependent phosphorylation was studied in male SHK mice irradiated with 800 rads. Animals were inoculated ip with labeled inorganic phosphate, dibutyryl-cAMP, and isoproterenol. Fractionation of the neutral nonhistone proteins from the thymus, spleen and liver showed that irradiation caused a 1.5-2x decrease of the level of cAMP-dependent phosphorylation of the thymus proteins in the spleen. Irradiation affected only the basic proteins with isoelectric point of 8.9-9.2. The decrease of adenylate cyclase activity was associated with the increase of phosphodiesterase activity in the spleen and an increase of phosphodiesterase activity in the thymus. (10 Refs) (In RUS)

32. ALTERED EPIDERMAL GROWTH FACTOR (EGF)-STIMULATED PROTEIN KINASE ACTIVITY IN VARIANT A431 CELLS WITH ALTERED GROWTH RESPONSES TO EGF.

Buss JE, Kudlow JE, Lazar CS, Gill GN
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Proc Natl Acad Sci USA; 79(8,part1):2574-2578 1982

To determine the role of epidermal growth factor (EGF)-stimulated protein kinase in the biological effects caused by EGF, tyrosine-specific kinase activity has been quantitated in A431 human epidermoid carcinoma cells and six variant cell lines. Because EGF inhibited proliferation of A431 cells, variants resistant to this inhibition were selected by treatment with mutagen and maintenance for 1 month in 0.1 uM EGF. After cloning and growth for 6-20 generations without EGF, the resistance of the variants to the growth-inhibitory effect of EGF was confirmed. Whereas EGF increased cellular phosphotyrosine content approx 10-fold in parental A431 cells, EGF caused smaller or undetectable increases in the six variant cell lines. Solubilized membranes from the six variants displayed diminished EGF-stimulated phosphorylation of the EGF receptor and of antibodies to p60src (the product of the Rous sarcoma virus transforming gene), which act as an exogenous substrate. The decrease in EGF-stimulated tyrosine-specific protein kinase activity varied from approx 40% (Clone 16) to approx 8% (Clone 18) of parental A431 activity. Phosphorylated EGF receptors from parental and variant cells migrated identically on sodium dodecyl sulfate/polyacrylamide gels. The number of EGF receptors in variant cells decreased in parallel with EGF-stimulated protein kinase activity, so that the specific activity of EGF-stimulated protein kinase per EGF receptor remained constant in the six variant cell lines with reductions in both activities to as low as 10%. These results suggest that this tyrosine-specific protein kinase activity mediates the growth-inhibitory effect of EGF on A431 cells and that both EGF binding and kinase activities reside in the same or tightly associated molecules. (Author abstract) (30 Refs)

33. INFLUENCE OF 3-METHYLCHOLANTHRENE ON LIVER NUCLEOLAR AND NUCLEOPLASMIC ACTIVITIES OF PROTEIN KINASES AND RNA POLYMERASES.

Kleeberg U, Szeberenyi J, Juhasz P, Tigyi A, Klinger W
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Biochem Pharmacol; 31(6):1063-1067 1982

The experiments were designed to investigate some details of the action of 3-methylcholanthrene (3-MC) on the regulation of transcription. After a single intraperitoneal dose of 3-MC a significant increase in the activities of both nucleolar and nucleoplasmic protein kinases in hepatic cells of young rats was found. The maximal stimulation took place 24 hr after the administration of 3-MC and the extent of activation was much greater in the nucleolar fraction. There is a significant elevation of the activities of both functional forms, free and template-engaged, of RNA polymerase A 24 hr after a single injection of 3-MC. Free and engaged forms of extranucleolar RNA polymerase B show a different behavior: after 24 hr of 3-MC administration the engaged form is markedly enhanced while the activity of the free enzyme shows a significant decrease. The more moderate increase in total RNA polymerase B activity is obviously preceded by a transfer of the enzyme from 'free' to 'engaged' form. Since the enhancement of protein kinase activities was accompanied by the stimulation of nuclear RNA polymerases we suggest that both kinds of enzymes are involved in an epigenetic mechanism of the inducing action of 3-MC on cytochrome P1-450. (Author abstract) (47 Refs)

34. AN EFFECT OF FIBRONECTIN IN EPIDERMAL GROWTH FACTOR (EGF)-STIMULATED MEMBRANE PROTEIN PHOSPHORYLATION IN HUMAN CARCINOMA CELLS.

Rieber M, Rieber MS
Centre Microbiology and Cell Biology, Instituto Venezolano de Investigaciones Cientificas, Caracas 1010-A, Venezuela
Exp Cell Res; 138(2):441-446 1982

Membrane components of about 20 kd (kilodalton) which appear weakly phosphorylated in protein kinase assays of A431 human carcinoma plasma membrane to which epidermal growth factor (EGF) is added, increase their relative phosphorylation when human serum fibronectin is also included in the assay. Membranes prepared from 32PO4-labeled cells exposed to EGF and fibronectin prior to organelle isolation revealed the presence of an anodic 20 kd component not detected in cells which were not exposed to fibronectin. No comparable effects were apparent with bovine heart protein kinase and with membrane-associated protein kinase preparations which were not responsive to EGF. (Author abstract) (17 Refs)

35. IDENTIFICATION OF A FISH PROTEIN ASSOCIATED WITH A KINASE ACTIVITY AND RELATED TO THE ROUS SARCOMA VIRUS TRANSFORMING PROTEIN.

Barnekow A, Schartl M, Anders F, Bauer H
Institut fur Virologie, Fachbereich Humanmedizin, Justus-Liebig-Universitat Giessen, Frankfurter Strasse 107, D-6300 Giessen, W. Germany
Cancer Res; 42(6):2429-2433 1982

Cells of all vertebrates thus far investigated contain a cellular src gene, which appears to be a highly conserved homolog of the Rous sarcoma virus oncogene src. Similar to the Rous sarcoma virus oncogene src, this endogenous cellular src gene codes for a Mr 60,000 phosphoprotein (pp60c-src) which is associated with a kinase activity. This kinase specifically phosphorylates tyrosine residues of proteins. We have systematically looked for pp60c-src in specific strains of certain teleostean fish, including the genera Xiphophorus and Girardinus. These strains are known to contain chromosomal oncogenes causing neoplastic transformation in certain mutants and hybrids. Since the pp60c-src found in other species has been shown to be antigenically related to the viral src gene product, a phosphoprotein with a molecular weight of 60,000 (pp60r-src), we have used tumor-bearing rabbit serum which recognizes Rous sarcoma virus pp60src to detect such a related protein in fish. Tumor-bearing rabbit serum precipitated two proteins with molecular weights of 60,000 and 52,000. The Mr 60,000 phosphoprotein was associated with a protein kinase activity that phosphorylates tyrosine residues of the immunoprecipitating immunoglobulin G heavy chain (Mr 53,000). The antigenic relationship of this fish protein to viral pp60src was further documented by an immune competition assay. We conclude, therefore, that the fish Mr 60,000 phosphoprotein is a pp60c-src encoded for by a fish cellular src gene that corresponds to the viral src oncogene. The Mr 52,000 phosphoprotein is probably a proteolytic degradation product of pp60c-src. All animals of the various strains of the fish investigated in this study showed kinase activity that was reactive upon immunoprecipitation with tumor-bearing rabbit serum, indicating that the pp60c-src protein is a normal constituent of the fish. Measurable quantitative differences were revealed between the various strains as well as in different tissues. Whereas brain and melanoma tissue showed a high kinase activity when compared to skin, liver, spleen, and testes, muscle tissue displayed no significant activity. (Author abstract) (27 Refs)

36. A CELLULAR PROTEIN IS IMMUNOLOGICALLY CROSSREACTIVE WITH AND FUNCTIONALLY HOMOLOGOUS TO THE FUJINAMI SARCOMA VIRUS TRANSFORMING PROTEIN.

Mathey-Prevot B, Hanafusa H, Kawai S

Rockefeller Univ., New York, NY, 10021

Cell; 28(4):897-906 1982

We obtained a regressing-tumor antiserum specific for the unique sequence of the transforming protein P140 of Fujinami sarcoma virus by injecting Fischer rats with syngeneic embryo cells transformed with Fujinami sarcoma virus. This serum is capable of immunoprecipitating a protein of 98,000 daltons from cell extracts of normal, uninfected chicken bone marrow cells. This normal cellular protein (NCP98) was shown to be structurally related to P140, sharing the majority of 35S-methionine-labeled tryptic peptides with the viral gene product P140. NCP98 is a phosphoprotein in vivo, with an association in vitro protein kinase activity, capable of phosphorylating specifically at tyrosine residues of NCP98 itself and alpha-casein, an externally added substrate. This kinase activity is biochemically indistinguishable from the kinase activity is biochemically indistinguishable from the kinase activity associated with P140 by all criteria tested. Moreover, in vitro-phosphorylated NCP98 and P140 shared the same phosphopeptides. The expression of NCP98 is tissue-specific. It is readily detectable in bone marrow cells and detectable to a lesser extent in liver and lung cells from 6-18 day old chickens. (Author abstract) (44 Refs)

37. SELECTIVE INHIBITION OF A CYCLIC NUCLEOTIDE INDEPENDENT PROTEIN KINASE (G TYPE CASEIN KINASE) BY QUERCETIN AND RELATED POLYPHENOLS.

Cochet C, Feige JJ, Pirollet F, Keramidas M, Chambaz EM

Biochimie Endocrinienne, INSERM U-244, CNRS ERA 942, Universite Scientifique et Medicale, CERMO, BP 53 X, 38041 Grenoble, France

Biochem Pharmacol; 31(7):1357-1361 1982

The effect of quercetin and a number of structurally related phenolic compounds upon the activity of three different purified protein kinases was examined. Whereas the catalytic subunit of a cyclic AMP-dependent protein kinase and an A type (using only ATP) cyclic nucleotide-independent casein kinase (CKA) were not affected, a G type (using GTP as well as ATP) casein kinase (CKG) was selectively inhibited by several bioflavonoid structures. Kinetic studies showed that quercetin behaved as a competitive inhibitor toward the nucleotidic substrate and exhibited a high affinity for the ATP (Ki = 0.75 uM) and GTP (Ki = 0.22 uM) site of the enzyme. Considering the CKG inhibitory potency of a series of flavonoid, cinnamic acid and coumarin derivatives, it is suggested that the biological activity lays upon a common structural feature involving a phenolic ring bearing a side chain with conjugated double bonds and an oxygenated function, as found in the coumaroyl residue. These observations suggest that quercetin and related compounds may lead to a shift in intracellular protein phosphorylations by selectively inhibiting a particular type of protein kinase activity (CKG). It remains to be established whether this process may contribute to the mechanism of action of flavonoids upon cellular metabolism, particularly in the case of malignant cells. (Author abstract) (15 Refs)

38. UTILIZATION OF GAMMA-32P-GTP BY ECTOPROTEIN KINASE OF 3T3 AND SV40 3T3 CELLS.

Mastro AM, Sniezek MJ

Dept. Microbiology, Cell Biology, Biochemistry and Biophysics, Pennsylvania State Univ., University Park, PA, 16802

Cell Biol Int Rep; 6(4):385-392 1982

The utilization of GTP by the membrane ectoprotein kinase of 3T3 and SV40-transformed 3T3 cells (SV40 3T3) was investigated. Gamma-32P-GTP was utilized by 3T3 about 77% of that seen with ATP. The reaction was cyclic AMP and cyclic GMP-independent and required Mg ions for activity. A comparison of the phosphorylation of quiescent 3T3, serum-stimulated 3T3, and SV40 3T3 cells showed the same relative extent of phosphorylation by ATP and GTP: SV40 3T3 greater than stimulated 3T3 greater than quiescent 3T3. In each case, the amount of incorporation with GTP was less than that with ATP. Proteins from cells phosphorylated with labeled GTP or ATP were separated by polyacrylamide gel electrophoresis. Radioautography showed generally similar labeling patterns with the two nucleotides. However, quiescent 3T3 cells exhibited a greater than 165,000 dalton protein that was labeled more extensively with ATP than with GTP. This band might represent more than one protein. The equivalent band in serum-stimulated cells was labeled to the same extent with ATP or GTP, although not as pronounced as in the quiescent cells. The rest of the banding patterns were similar to those of the quiescent cells. Thus, the 3T3 and SV40 3T3 cell ectokinase is a membrane-related to cyclic AMP-independent enzyme which can utilize GTP as well as ATP to phosphorylate similar substrates. (19 Refs)

39. CHANGES IN CYCLIC AMP-DEPENDENT PROTEIN KINASES DURING INHIBITION OF MASTOCYTOMA CELL GROWTH BY DIBUTYRYL CYCLIC AMP.

Evans J, Smart J, Airey P, Ralph R
Dept. Cell Biology, Univ. Auckland, Auckland, New Zealand
Mol Cell Biochem; 43(3):183-190 1982

Inhibition of growth of PY815 mouse mastocytoma cells in vitro by N6,O2'-dibutyryladenosine 3',5' cyclic monophosphate (DB cyclic AMP) was accompanied by increases in intracellular cyclic AMP and histamine and minor changes in cytosolic cyclic AMP-dependent histone kinase activity. However, DEAE-cellulose chromatography revealed substantial changes in the relative proportions of the principal cyclic AMP-dependent protein kinases and in free cyclic AMP-binding protein after DB cyclic AMP treatment. The activity of cytosolic cyclic AMP-dependent protein kinase Type I (PK1) decreased relative to cyclic AMP-dependent protein kinase Type II (PKII) and there was an increase in a cytosol cyclic AMP-binding protein with little associated protein kinase activity. The relative changes in activity of PKI, PKII and cyclic AMP binding protein after DB cyclic AMP treatment may reflect events important in the regulation of growth and differentiation of mast cells. (Author abstract) (43 Refs)

40. TRANSFORMATION BY AVIAN SARCOMA VIRUSES LEADS TO PHOSPHORYLATION OF MULTIPLE CELLULAR PROTEINS ON TYROSINE RESIDUES.

Beemon K, Ryden T, McNelly EA
Dept. Biology, Johns Hopkins Univ., Baltimore, MD, 21218
J Virol; 42(2):742-747 1982

Phosphoamino acid compositions were determined for 10 size classes of cellular proteins, separated by electrophoresis through one-dimensional sodium dodecyl sulfate-polyacrylamide gels. Phosphotyrosine-containing proteins were observed in uninfected chicken embryo fibroblasts in every size class analyzed, ranging from approx 20,000 to greater than 200,000 daltons. Transformation of chicken embryo fibroblasts by Rous sarcoma virus or PRC II avian sarcoma virus led to increases in phosphorylation of proteins at tyrosine residues in all of these size classes. A large fraction of the phosphotyrosine-containing protein molecules observed in Rous sarcoma virus-transformed cells was larger than 100,000 daltons with a second broad peak in the 35,000-to 60,000-dalton range. This study suggests that there are a number of substrates of viral or cellular tyrosine-specific protein kinases, which have not yet been identified by other methods. (Author abstract) (31 Refs)

41. AFFINITY LABELING OF THE PROTEIN KINASE ASSOCIATED WITH THE EPIDERMAL GROWTH FACTOR RECEPTOR IN MEMBRANE VESICLES FROM A431 CELLS.

Buhrow SA, Cohen S, Staros JV
(c/o Staros) Dept. Biochemistry, Vanderbilt Univ. Sch. Medicine, Nashville, TN, 37232
J Biol Chem; 257(8):4019-4022 1982

Epidermal growth factor (EGF), a mitogenic polypeptide hormone, stimulates the phosphorylation of certain endogenous proteins in membrane preparations derived from A431 cells, a human tumor cell line. Membrane vesicles prepared from A431 cells were reacted with 5'-p-fluorosulfonylbenzoyl adenosine (5'-p-FSO2BzAdo). Reaction of the vesicles with 5'-p-FSO2BzAdo results in a time-dependent inhibition of EGF-stimulable protein kinase activity which parallels an increase in incorporation into the vesicles of the 5'-p-sulfonylbenzoyl-[8-14]adenosine moiety from 5'-p-FSO2Bz[14C]Ado. The primary bands labeled have Mr = 170,000 and 150,000. Labeling of these bands by 5'-p-FSO2Bz[14C]Ado is inhibited by incubation of the membrane vesicles with adenyl-5'-yl imidodiphosphate, an ATP analog. Inactivation of the kinase with N-ethylmaleimide or by heating results in a sharply decreased labeling of the proteins with Mr = 170,000 and 150,000. Proteins of these mol wt have previously been identified in these cells as the EGF receptor and a degradation product of the receptor. These experiments provide chemical evidence that the EGF receptor and the EGF-stimulable kinase are the same protein. (Author abstract) (36 Refs)

42. TRANSFORMATION INDUCED BY ABELSON MURINE LEUKEMIA VIRUS INVOLVES PRODUCTION OF A POLYPEPTIDE GROWTH FACTOR.

Twardzik DR, Todaro GJ, Marquardt H, Reynolds FH, Stephenson JR

Lab. Viral Carcinogenesis, NCI, Frederick, MD, 21701

Science; 216(4548):894-897 1982

Rat embryo fibroblast transformed by Abelson murine leukemia virus (MuLV) produce and release a transforming growth factor (TGF). Production of this factor is correlated with a tyrosine-specific protein kinase that is functionally active and is associated with the major Abelson MuLV gene product, P120. Transformation-defective mutants of Abelson MuLV do not transform cells, do not have their virus coded transforming gene product phosphorylated in tyrosine, and do not induce TGF production. Abelson MuLV-induced TGF morphologically transforms cells in culture, competes with 125I-labeled epidermal growth factor (EGF) for binding to cell receptors, and induces phosphorylation of tyrosine acceptor sites in the 160,000-dalton EGF membrane receptor. After purification to homogeneity, Abelson virus-induced TGF migrates as a single polypeptide with an apparent size of 7,400 daltons as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. (Author abstract) (27 Refs)

43. VIRAL SRC GENE PRODUCTS ARE RELATED TO THE CATALYTIC CHAIN OF MAMMALIAN CAMP-DEPENDENT PROTEIN KINASE.

Barker WC, Dayhoff MO

Natl. Biomedical Res. Foundation, Georgetown Univ. Medical Center, Washington, DC, 20007

Proc Natl Acad Sci USA; 79(9):2836-2839 1982

The transforming protein sequences translated from the Rous avian and Moloney murine sarcoma virus src genes are shown to be related to the catalytic chain of bovine cAMP-dependent protein kinase (ATP: protein phosphotransferase, EC 2.7.1.37). The avian transforming protein, also a protein kinase, shows greatest homology with the bovine protein kinase in the carboxyl-terminal half, where the protein kinase activity is localized. Moreover, lysine occurs in the inferred transforming protein sequences at the position homologous with the proposed ATP-binding lysine of the bovine protein kinase. This relationship is consistent with the hypothesis that the src genes originated in the host genomes, in which they are members of a superfamily of distantly related protein kinases that are normal constituents of mammalian cells. In the host, these sequences are much more highly conserved than in the viruses. (Author abstract) (16 Refs)

44. SYNTHESIS AND TURNOVER OF REGULATORY SUBUNIT OF PROTEIN KINASE I IN NEUROBLASTOMA CELLS (MEETING ABSTRACT)

Prashad N
Graduate School of Biomedical Sciences, University of Texas Health Science Center, Houston, TX, 77025
Fed Proc; 41(3):659 1982

Multiple forms of regulatory subunit of PKI (R1 and R) exist in mouse neuroblastoma cells. R1 and R have a MW of 48,000; however, the PI are different, R1 (PI 5.2) and R (PI 5.4). DibutyrylcAMP increased R by 3-fold without affecting R1. Pulse labeling of cells with 35(s)met for 5 min, show an increase in R by 2-fold in dibutyrylcAMP treated cells compared to control cells, suggesting increase in R is due to de novo synthesis. Translation of 1ug of poly(A)+RNA in reticsys show a 2-fold increase in the synthesis of R by poly(A)+RNA from dibutyrylcAMP treated cells compared to control cells. These results indicate dibutyrylcAMP-induced R is due to increase in the specific mRNA, suggesting different mechanisms for the synthesis of R1 and R. The turnover rates of R1 and R were determined by chasing 35(S)met labeled cells in the absence and presence of dibutyrylcAMP. The half-life of R in the absence of dibutyrylcAMP was 25 hr and in the presence of dibutyrylcAMP R was stabilized, decreasing 16% after 48 hr. In contrast, R1 was stable in the absence or presence of dibutyrylcAMP, decreased by 23% after 48 hr. These data show R1 and R have different turnover rates. Therefore, dibutyrylcAMP induced R, due to increased synthesis as well as increased stability due to R-cAMP complex formation. Characterization and understanding the functions of multiple forms of regulatory subunit will help to understand their role in cellular functions, especially in dibutyrylcAMP-induced differentiation. (no Refs)

OTHER RELATED STUDIES

45. ANTAGONISM OF CONCANAVALIN A CAPPING IN PHORBOL ESTER-ACTIVATED LYMPHOCYTES BY CALMODULIN INHIBITORS AND CERTAIN AMINO ACID ESTERS.

Kwong CH, Mueller GC
(c/o Mueller) McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI, 53706
Cancer Res; 42(6):2115-2120 1982

The compound 12-O-tetradecanoylphorbol-13-acetate (TPA), an effective tumor promoter in mouse skin and comitogen in bovine lymphocytes, rapidly stimulates concanavalin A-mediated cap formation in the latter cells. The ability of different phorbol derivatives to facilitate the capping reaction correlates well with their potencies as lymphocyte comitogens and as tumor-promoting agents. This effect of TPA on capping in bovine lymphocytes, which is apparent within min, is neither mimicked nor altered by dibutyryl cyclic adenosine 3':5'-monophosphate or cyclic guanosine 3':5'-monophosphate. Cytochalasin D, a microfilament-disrupting agent, inhibits cap formation, thereby suggesting the participation of microfilaments in the response. benzoyl tyrosine ethyl ester selectively inhibits the TPA-stimulated cap formation, whereas benzoyl tyrosinamide is inactive. A comparison of related amino acid derivatives reveals that their activities are dependent on the nature of both the amino acid side chain and the carboxyl end blocking group. Trifluoperazine and N-(6-aminohexyl)-5-chloronaphthalenesulfonamide, known inhibitors of the calmodulin-dependent processes, also selectively block the TPA-stimulated cap formation, whereas trifluoperazine sulfoxide, a less effective calmodulin antagonist, is relatively inactive. These data suggest that the stimulation of capping by TPA involves the activation of a calmodulin-dependent process which may also be regulated by the function of an esterase. (Author abstract) (32 Refs)

46. PHENOTHIAZINE EFFECTS ON GONADOTROPIN-RESPONSIVE LEYDIG TUMOR CELLS (MEETING ABSTRACT)

Melner MH, Zimniski SJ, Puett D
Department of Biochemistry, Vanderbilt University, Nashville, TN, 37232
Fed Proc; 41(3):885 1982

These studies were undertaken to examine the effects of two phenothiazines, chlorpromazine (CP) and trifluoperazine (TFP), on adenylate cyclase (AC) activity and steroidogenesis in murine M5480A Leydig tumor cells. TFP is a potent inhibitor of many calmodulin-mediated processes, while CP has a much lower efficacy. Using dispersed Leydig tumor cells in primary culture, TFP (0.1-25 uM) inhibited both basal and human choriogonadotropin (hCG)-stimulated testosterone production. In contrast, CP at similar doses stimulated testosterone production. Moreover, the effects of the combination of CP and a maximal dose of hCG were additive in terms of androgen production. In particulate membrane fractions, TFP inhibited 5'-guanylimidodiphosphate [Gpp(NH)p]-stimulated AC activity both with and without hCG. On the other hand, CP stimulated AC activity in the presence of Gpp(NH)p, but did not alter the enzymic activity in the presence of maximal stimulatory doses of Gpp(NH)p (10 uM) and hCG (0.1 uM). Thus, in general the effects of the drugs on AC activity paralleled those in steroidogenesis. It is noteworthy that the two phenothiazines exhibit quite different actions on these two parameters and may prove useful in elucidating regulatory mechanisms in steroidogenesis. (no Refs)

47. DEFERRED PARATHYROID AUTOGRAFTS WITH CRYOPRESERVED TISSUE AFTER REOPERATIVE PARATHYROID SURGERY.

Saxe AW, Spiegel AM, Marx SJ, Brennan MF
(c/o Brennan) Dept. Surgery, Memorial Sloan Kettering Cancer Center, 1275 York Ave., New York, NY, 10021
Arch Surg; 117(5):538-543 1982

Between August 1975 and March 1981, 12 patients underwent forearm parathyroid autotransplantation with cryopreserved tissue following reoperative parathyroid surgery. Tissue had been cryopreserved 2 to 18 mo. Follow-up ranged from 4 to 66 mo (median, 18 mo) following autotransplantation. All patients required calcium supplementation as a condition for autografting; six patients were no longer receiving calcium supplementation at most recent follow-up and in one patient the dosage was being tapered. The presence of a parathyroid hormone gradient of 2:1 or greater correlated well with freedom from calcium supplementation. Of seven patients with preautograft and postautograft urinary cyclic adenosine monophosphate (cAMP) determinations, three with increases following transplantation did not require calcium, three without increases continued to require calcium, and the patient whose dosage was being tapered had increased urinary cAMP levels. (Author abstract) (33 Refs)

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AUTHOR INDEX WITH CITATION NUMBERS

Abou-Issa H 15
Adkins J 23
Airey P 39
Aiyar NV 11
Ambrus CM 29
Ambrus JL 29
Anders F 35
Andersson RG 27
Axelsson KL 27
Bakkeren JA 28
Barker WC 43
Barnekow A 35
Bauer H 35
Beemon K 40
Biddle W 29
Binet E 7
Blume AJ 18
Boucek RJ 9
Brennan MF 47
Buhrow SA 41
Buss JE 32
Chambaz EM 37
Chen KY 5
Chirkov Iulu 31
Cho-Chung YS 22
Christoffersen 26
Clair T 22
Cochet C 37
Cohen S 41
Conlon-Hollings 2
Criss WE 23
Dayhoff MO 43
De Abreu RA 28
De Bruyn CH 28
Dhillon G 23
Estes NC 30
Evain D 7
Evans J 39
Fakunle J 23
Farmer JL 20
Feige JJ 37
Fishman PH 24
Fitton LA 25
Foecking MK 15
Ford CJ 21
Gastpar H 29
Gill GN 32
Gladhaug IP 26
Glover JL 30
Goldman PR 17
Gottesman NM 6
Halcak L 4
Hanafusa H 36
Henneberry RC 24
Honeysett JM 10
Horoszewicz J 29
Huberman E 29
Hunter GJ 25
Insel PA 10
Juhasz P 33
Kassis S 24
Kato H 3
Kato T 3
Kato T 3
Kawai S 36
Kenimer JG 13
Keramidas M 37
Kleeberg U 33
Klinger W 33
Knight E 23
Kudlow JE 32
Kwong CH 45
Lazar CS 32
Lembach KJ 9
Leong SS 29
Lichtstein D 18
Liu AY 5
MacLaren IA 16
Marquardt H 42
Marx SJ 47
Masada M 14
Masaoka A 3
Mastro AM 38
Mathey-Prevot B 36
Matsumoto M 1
McNelly EA 40
Melner MH 46
Miller D 30
Minton JP 15
Montagna R 29
Morris HP 23
Mueller GC 45
Mullikin-Kilpat 18
Murray AW 19
Naganawa N 3
Nagasawa S 1
Nahlik J 2
Nambi P 11
Negishi S 14
Obika M 14
Ohoka T 14
Ortwerth BJ 2
Parken N 5
Pastan I 6
Phillipps MA 21
Pirollet F 37
Prager MD 20
Prashad N 44
Prashad N 8
Presepe V 5
Psychoyos S 21
Puett D 46
Ralph R 39
Refsnes M 26
Reynolds FH 42
Rieber M 34
Rieber MS 34
Rogers PC 16
Roth CW 6
Rydell EL 27
Ryden T 40
Sachs L 12
Sakazaki Y 3
Sand TE 26
Saxe AW 47
Schartl M 35
Schwetlen ED 28
Seiki Y 1
Serbin P 31
Sharma RK 11
Shepheard C 22
Shibata I 1
Simantov R 12
Singh T 6
Skala JP 16
Smart J 39
Sniezek MJ 38
Sobolev AS 31
Solanki V 19
Spiegel AM 47
Staros JV 41
Stephenson JR 42
Svec J 4
Svec P 4
Swoboda BE 25
Szeberenyi J 33
Takezawa J 1
Tanaka R 3
Thurzo V 4
Tigyi A 33
Todaro GJ 42
Tsunooka H 3
Twardzik DR 42
Van Baal JM 28
Vogel WH 17
Wakamatsu Y 14
Watanabe A 30
Weiss J 9
Wikberg JE 27
Wolters J 2
Yamakawa Y 3
Zimniski SJ 46

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In this issue:

- **REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES** 1-4
- **REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES** 5-6
- **CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES** 7-16
- **CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS** 17-23
- **CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES** 24-25
- **PROTEIN KINASE STUDIES** 26-40
- **OTHER RELATED STUDIES** 41-43

REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES

1. INHIBITION OF HEPATOMA CELL GROWTH BY ANALOGS OF ADENOSINE AND CYCLIC AMP AND THE INFLUENCE OF ENZYMES IN MAMMALIAN SERA.

Hargrove JL, Granner DK
(c/o Granner) Dept. Internal Medicine, Univ. Iowa and Veterans Admin. Medical Center, Iowa City, IA, 52240

J Cell Physiol; 111(3):232-238 1982

Toxic effects of cyclic AMP (cAMP) and N6,O6-dibutyryl cAMP (Bt2cAMP) were studied in hepatoma tissue culture (HTC) cells, whose growth is not inhibited by Bt2cAMP, and Reuber H35 cells, whose growth is reduced by several analogs of cAMP. In both cell lines, Bt2cAMP exerts a positive effect by increasing tyrosine aminotransferase content. Conditions were examined under which toxicity of cyclic nucleotides and adenosine analogs occurred or was prevented when serum type or treatment was varied. HTC and H35 cells were grown in Swim's S-77 medium containing 5% fetal calf serum and 5% bovine serum. Growth of HTC cells in spinner culture was in-

HE 20.3173/2:

hibited in the presence of cAMP, 5'-AMP, or adenosine. Virtually no growth occurred when cAMP was present in medium containing serum heated for 20 min; in the absence of the cyclic nucleotide, cell numbers increased to 60,000/ml during 3 days of growth. Uridine and cytidine both prevented adenosine toxicity. The effects of isopentenyl adenosine on cell growth included inhibition of cell growth, nucleoside uptake, orthophosphate uptake, and DNA synthesis. Uptake of 2-deoxy-D-glucose and alpha-aminoisobutyric acid was not affected. Results suggested that toxicity of cAMP and Bt2cAMP in the two hepatoma lines is a nonspecific, adenosine-like effect attributable to metabolism of these compounds in the medium and within the cells. (44 Refs)

2. DIFFERENTIATION OF NEUROBLASTOMA CELLS BY CHICK GIZZARD EXTRACT.

Miki N, Higashida H, Hayashi Y, Akiyama M, Kuo CH

Dept. Pharmacology, Cancer Res. Inst., Kanazawa Univ., 13-1 Takaramachi, Kanazawa 920, Japan

Neurochem Int; 4(1):49-56 1982

Cholinergic neuroblastoma NS20Y cells were differentiated by a crude extract of chick gizzard (CGE). Biochemical and electrophysiological changes of the differentiated neuroblastoma cells, and properties of the CGE are described. Decreased cyclic guanosine monophosphate levels and increased membrane excitability were the principal changes exhibited by differentiated cells. (18 Refs)

3. INDUCTION OF NEOPLASTIC CELL GROWTH BY QUIESCENT CELLS IS MEDIATED BY SERUM CONCENTRATION AND CAMP PHOSPHODIESTERASE INHIBITORS.

Bertram JS, Bertram BB, Janik P

Grace Cancer Drug Center, Roswell Park Memorial Inst., Buffalo, NY, 14263

J Cell Biochem; 18(4):315-538 1982

Studies have shown that for transformed mouse fibroblasts (C3H/101/2) and Lewis lung carcinoma model systems, the expression of transformation is conditional upon the permissive behavior of neighboring cells. Such permissive behavior could be due to a failure in intercellular communication or to a failure in signal reception or processing. For a constant plating density an increase in serum concentration was shown to cause a dose responsive decrease in N-methyl-N-nitro-N-nitrosoguanidine (MNNG)-induced transformation frequency in fibroblast cells. This increase in serum concentration produced an increase in saturation density of confluent cells. A model is developed which suggests that interference in communication between cells occurs during transformation. The passage of small growth inhibitory substances through gap junctions between contacting cells controls cell cycle phase. Autonomous growth would occur through attenuation of signal intensity. This studies have identified two possible factors which may control signal strength, ie, cell density and elevated cAMP. The model thus suggests that transformed cells are deficient in their ability to form communication channels with themselves or with adjacent normal cells. The major effect of phosphodiesterase inhibitors may be to increase the latent period between tumor injection and the appearance of tumor. Applications of the model to phosphodiesterase inhibitors and to tumor promotion are discussed. (42 Refs)

4. EFFECTS OF RETINOIC ACID ON DIFFERENTIATION OF CHORIOCARCINOMA CELLS IN VITRO.

Chou JY

Pregnancy Res. Branch, Natl. Inst. Child Health and Human Development, NIH, Building 6, Room 128, Bethesda, MD, 20205

J Clin Endocrinol Metab; 54(6):1174-1180 1982

Choriocarcinoma cells maintain multiple hormonal functions in culture. We have found that these cells secreted no immunoreactive pregnancy-specific beta1-glycoprotein (PSbetaG), a placental protein. Choriocarcinoma cells can be induced to synthesize low levels of PSbetaG by retinoic acid, 8-bromo-cAMP (8BrcAMP), cholera toxin, methyl-isobutylxanthine (MIX), and 5-bromo-2'-deoxyuridine (BrdUrd). The simultaneous addition of retinoic acid along with 8BrcAMP, cholera toxin, or MIX gave synergistic induction of PSbetaG. The simultaneous addition of retinoic acid and BrdUrd failed to give even additive induction. In addition to stimulating PSbetaG production, retinoic acid increased the production of hCG and its alpha-subunit (hCGalpha) by choriocarcinoma cells. The simultaneous addition of retinoic acid along with 8BrcAMP, cholera toxin, or MIX gave additive induction for hCG and hCGalpha. Passage of choriocarcinoma cells in medium containing retinoic acid induced a stable altered phenotype characterized by elevated levels of PSbetaG, hCG, and hCGalpha. These retinoid-treated choriocarcinoma cells remained responsive to 8BrcAMP or compounds that increase intracellular cAMP concentrations and to BrdUrd; the production of PSbetaG and hCG was greatly stimulated by 8BrcAMP, cholera toxin, or MIX, and the production of hCG and hCGalpha was greatly inhibited by BrdUrd. However, the production of hCGalpha was only slightly induced by these cAMP modulators, and the production of PSbetaG was not increased by BrdUrd. (Author abstract) (29 Refs)

REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES

5. TAMOXIFEN INHIBITS LEYDIG CELL STEROIDOGENESIS: IN VIVO AND IN VITRO STUDIES.

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Metabolism; 31(6):543-547 1982

The effects and mechanism of action of tamoxifen (TAM) on steroidogenesis in Leydig cells of mature Sprague-Dawley rats were investigated. Hypophysectomized rats were treated with 0, 10, or 100 ug TAM suspended on 0.1 ml corn oil. Twenty-four hr later, tail blood samples were obtained, followed by administration of 5 units/100 g body wt human chorionic gonadotropin (HCG) sc. Two hr later, rats were sacrificed, trunk blood collected, and sera separated and stored. TAM in a dose of 10 ug had no effect on HCG-stimulated testosterone in 21 day old hypophysectomized rats formation, while 100 ug TAM did reduce HCG-stimulated testosterone synthesis significantly. TAM had no effect upon basal testosterone levels in 60 day old hypophysectomized rats. After HCG stimulation, there was a 46% reduction in mean plasma testosterone levels in rats treated with 10 ug TAM and 69% reduction in rats treated with 100 ug TAM. When decapsulated testes from these rats were incubated for 3 hr, rats treated with 100 ug TAM produced significantly less testosterone when compared to rats treated with either 10 ug TAM or controls. TAM thus directly inhibits testosterone response to gonadotropin stimulation both in immature and mature hypophysectomized rats. Conversion of pregnenolone to testosterone was blocked by TAM. Both estrogen and TAM inhibited leuteinizing hormone-stimulated testosterone formation, but with different patterns of inhibition. TAM had no effect upon HCG binding to gonadotropin receptors, or upon adenylate cyclase activity. These results indicate that TAM blocks multiple steps in steroidogenesis. (29 Refs)

6. SEQUENTIAL ASPECTS OF CHEMICAL CARCINOGENESIS: SKIN.

Berenblum I
Weizmann Inst. Science, Rehovot, Israel
Cancer - A Comprehensive Treatise Vol. 1. Etiology - Chemical and Physical Carcinogenesis. Second Edition. Becker FF, ed. New York, Plenum Press, 714 pp., 1982.

The two-stage hypothesis, the initiation-promotion hypothesis, was based on animal studies of sequential stages of skin carcinogenesis. Initiators for skin carcinogenesis include 2-acetylaminofluorene, UV light, beta-radiation, certain metabolites of 'complete' carcinogens, various alkylating agents and mutagens. Promoters for skin carcinogenesis include physical agents, 'complete' chemical carcinogens, non- or weakly-acting skin carcinogens, croton oil, straight-chain N-alkanes, phenolic compounds, extracts of tobacco leaf, and extracts of tobacco smoke condensates. The two-stage system in skin carcinogenesis has been extended, based on further experiments. Evidence that a mutational change in the DNA genome was responsible for the initiating change of carcinogenesis is based on the ability of most carcinogens to bind with DNA and that there is a fairly good correlation between mutagenic and carcinogenic properties among a wide range of compounds. Evidence for the stimulation of synthetic processes in the cell includes the stimulation of DNA and RNA synthesis as well as protein and phospholipid synthesis. The effects of enzymatic functions in the cell are based on an increase in proteinase activity, on stimulation of ornithine decarboxylase activity, on changes in cyclic nucleotide levels, and on a decrease in histidase activity. (378 Refs)

CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES

7. THE INCREASE IN HORMONE-STIMULATED ADENYLATE CYCLASE ACTIVITY FOLLOWING ROUS SARCOMA VIRUS TRANSFORMATION.

Howard RF, Friis RR, Sheppard JR, Glossmann H
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J Cell Physiol; 111(3):295-302 1982

Ability of prostaglandin E1 (PGE1) and isoproterenol (ISP) to elevate the intracellular concentration of cyclic AMP (cAMP) was studied in uninfected cells, cells infected and transformed by Rous sarcoma virus (RSV) and Rous associated virus (RAV). Uninfected chicken embryo cells (CEC), cells infected but not transformed by RAV, and RSV-transformed cells in culture immediately synthesized increased amounts of cAMP upon exposure to 10 uM ISP, 10 uM PGE1, or prostaglandin E2. RSV-transformed cells were consistently more responsive to maximal stimulatory concentrations of ISP than uninfected and RAV-infected cells. RSV-transformation was observed to alter the adenylate cyclase activity measured by CEC membrane preparations. The time courses for the hormone stimulation of intact RSV-transformed and RAV-infected cells suggested that the untransformed cells were rapidly desensitized during continued beta-adrenergic receptor stimulation, while the transformed cell cultures continued to respond to ISP. Results indicated that transformed cells possess a greater number of beta-adrenergic receptors, and are more slowly desensitized by stimulatory concentrations of ISP. Membranes prepared from transformed cells specifically bound greater than 5x the quantity of radiolabeled (-)3H-dihydroalprenolol and 125I-iodocyanopindolol as compared with the untransformed cell membranes suggesting that the major difference in the untransformed and transformed cells is in the concentration of membrane beta-adrenergic receptors and the inability of RSV-transformed cells to self-limit their response to specific external stimuli. (43 Refs)

8. HYPOTHESIS: CYCLIC AMP TURNOVER IN S49 CELLS.

Barber R, Goka TJ
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J Cyclic Nucleotide Res; 7(6):353-361 1981

A simplified method for estimating cyclic AMP (cAMP) turnover in S49 lymphoma cells is reported. The method assumes that for tritium label incorporation into cAMP upon treatment of cells with [3H]-adenine, the label must first pass through ATP. An equation was devised for relating the rate of change in cAMP specific radioactivity with cAMP and ATP specific radioactivities. A linear graphical plot with the fractional turnover constant as the slope of the line was obtained. In S49 wild type cells, the fractional turnover constant was estimated at 0.083/min, equivalent to a half life for cAMP of approx 8 min. This value was confirmed when cells were stimulated with epinephrine (100 nanoM) for 40 min at 24 C and then exposed to 10 uM propranolol. cAMP was determined at various times thereafter. The decay constant was measured as 0.093/min, similar to 0.083/min determined by pulse labeling. When turnover constants were greater than 1.0/min, the values could not be determined with accuracy by this technique. The key step in the experimental procedure was separation of ATP from cAMP by column chromatography. The decay and turnover constants in S49 cells differed significantly from those obtained with a variety of cultured fibroblasts. (12 Refs)

9. CYCLIC AMP BINDING PROTEINS, OESTROGEN RECEPTORS AND PROGESTERONE RECEPTORS IN HUMAN BREAST CANCER (MEETING ABSTRACT)

Watson D, Hawkins RA, Senbanjo R, Telford J, Forrest AP
Department of Clinical Surgery, University of Edinburgh, Edinburgh, Scotland, Miller, W. R.
Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh, Scotland. British Association for Cancer Research 78 pp., 1982.

While human breast cancer which possess estrogen receptor (ER) and progesterone receptor (PgR) activities are likely to be hormone responsive, many do not respond to endocrine therapy. In hormone-dependent rat mammary tumors the ratio of cyclic AMP binding proteins to steroid receptors may better discriminate hormone dependent from independent tumors than steroid receptors alone. The inter-relationships between cyclic AMP binding protein, ER and PgR activities have therefore been determined in 100 human cancers. Cyclic AMP binding was detected in all tumors, ER in 70 and PgR in 38. Mean cyclic AMP binding level in ER negative tumors was significantly higher than in the ER positive group. In tumors with both activities there was no significant correlation between levels of binding. Similar relationships were also found between cyclic AMP binding and PgR. Expressing results as ratios of ER to cyclic AMP binding proteins neither divided the tumors into obvious subgroups nor discriminated between PgR positive and negative tumors. (no Refs)

10. PROGNOSTIC VALUE OF CYCLIC AMP BINDING PROTEIN IN HUMAN BREAST CANCER (MEETING ABSTRACT)

Senbanjo RO, Miller WR, Telford J, Watson D, Forrest AP
Department of Clinical Surgery, University of Edinburgh, Edinburgh, Scotland
Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh, Scotland. British Association for Cancer Research 78 pp., 1982.

Cyclic adenosine -3'5' - Monophosphate (cyclic AMP) and its binding proteins are involved in regulating the growth of mammary tumors in experimental animals. In the present study, we have investigated the relationship between cyclic AMP binding proteins in human breast cancers and clinical parameters including prognosis. The binding proteins were measured in the cytosols from 75 human breast cancers. All tumors contained measurable binding proteins, levels varying from 0.81 to 15.05 omols/mg cytosol protein. No relationship was found between level of activity and menopausal status of the patient, clinical stage of the disease, presence or absence of nodal involvement or histological grade of the tumor. A group of 11 patients who at the time of primary treatment had no evidence of metastatic disease but have developed recurrence within 36 months have been compared with a similar group (10 patients) who have had a minimum of 20 months follow-up and were disease-free. Levels of cyclic AMP binding proteins was significantly higher in tumors from women having early recurrence than in those who were disease-free. The range in disease-free patients was 1.57-7.21 omols/mg cytosol protein; whereas that in 10 of 11 tumors with subsequently recurred early was 7.75-13.02. (2 Refs)

11. POLYPLOID RAT GLIOMA CELLS. PRODUCTION, OSCILLATIONS OF MEMBRANE POTENTIAL AND RESPONSE TO NEUROHORMONES.

Heumann R, Reiser G, Van Calker D, Hamprecht B
Max-Planck-Institut fur Psychiatrie, 8033 Martinsried, W. Germany
Exp Cell Res; 139(1):117-126 1982

Due to their small size, electrophysiological investigation of C6 rat glioma cells by intracellular recording with microelectrodes is very difficult. In order to facilitate such electrophysiological studies, the degree of ploidy of C6 cells was increased in three successive steps. At each step complementary drug-resistant mutants of C6 were fused and hybrid cell clones isolated in selective culture medium. Cell volume was measured by electronic cell sizing and DNA content per cell assessed by impulse cytofluorimetry. In the final polyploid line, C6-4-2, both parameters were found to be increased sixfold in comparison with the original cell line C6. In response to beta-adrenergic agonists and prostaglandin E1 the intracellular concentration of cAMP was raised in C6-4-2 cells. Stable membrane potentials could be recorded from the polyploid glioma cells much more easily than from the original C6 cells. The plasma membrane of the C6-4-2 cells could hyperpolarize spontaneously with the a frequency of about 1 cycle/min. Elevation of the concentration of Ca++ ions (but not other earth alkali ions) in the medium induced the onset of the spontaneous hyperpolarizations. The membranes of the C6-4-2 cells also hyperpolarized in response to acetylcholine, gamma-aminobutyric acid (GABA) and glutamate. (Author abstract) (51 Refs)

12. LIPOMATOSIS: POSSIBLE MODELS OF METABOLIC OBESITY IN HUMANS?.

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Inn Med; 9(3):96-98 1982

The response of lipomatosis to catecholamines was studied. Nodular lipomas and normal tissue controls were obtained under local anesthesia from five men (30-50 yr old) with nodular encapsulated lipomatosis (NEL). None of the patients had metabolic disease or abnormal hepatic function. Tissue samples were obtained by needle biopsy from two patients (45 and 55 yr old) with multiple symmetric lipomatosis (MSL). Both patients had histories of at least 10 yr of alcohol abuse. MSL had existed for 10 yr in one patient and 2 yr in the other. One patient showed abnormal liver function with elevated transaminase activity and GGT activity. The other patient had only an elevated GGT activity. The response of adenylate cyclase to catecholamines was studied in preparations of disrupted cells from the patients with NEL and in preparations of intact fat cells from patients with MSL. Dose-effect curves for response to adrenalin were identical for normal and neoplastic preparations from the NEL patients, indicating that the key enzyme for the catecholamine effect exhibited a normal beta-adrenergic response in both tissue types. A defect at the level of catecholamine receptors in NEL was therefore ruled out. Isoproterenol elicited identical maximum responses in control cells and MSL cells. Half-maximum effects were obtained in controls with a concentration of 0.3 uM isoproterenol, compared with 3.0 uM in MSL cells. Apparently hyperplasia of fatty tissues soes not result from resistance to catecholamines in NEL or MSL cells. (12 Refs) (In GER)

13. DIRECT MODIFICATION OF THE MEMBRANE ADENYLATE CYCLASE SYSTEM BY ISLET-ACTIVATING PROTEIN DUE TO ADP-RIBOSYLATION OF A MEMBRANE PROTEIN.

Katada T, Ui M

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Proc Natl Acad Sci USA; 79(10,part1):3129-3133 1982

GTP and isoproterenol activation of adenylate cyclase [ATP pyrophosphate-lyase (cyclizing) EC 4.6.1.1] in washed membranes prepared from C6 glioma cells was enhanced by incubation with islet-activating protein, one of the pertussis toxins, if the incubation mixture was supplemented with NAD and ATP. The action of the protein was observed immediately after its addition and increased progressively in magnitude as the protein concentration or the incubation time increased. There was simultaneous incorporation of radioactivity from the ADP-ribose moiety of variously labeled NAD into the membrane protein with a mol wt of 41,000. We conclude that islet-activating protein enhances receptor-mediated GTP-induced activation of membrane adenylate cyclase as a result of ADP-ribosylation of a membrane protein, probably one of the components of the receptor-adenylate cyclase system. (Author abstract) (21 Refs)

14. HYPERCALCEMIA ASSOCIATED WITH GYNECOLOGIC MALIGNANCIES. BIOCHEMICAL CHARACTERIZATION.

Stewart AF, Romero R, Schwartz PE, Kohorn EI, Broadus AE
Yale Univ. Sch. Medicine, LMP 2074, 333 Cedar St., New Haven, CT
Cancer; 49(11):2389-2394 1982

Fasting calcium excretion, renal phosphorus threshold, plasma 1,25-dihydroxyvitamin D, immunoreactive PTH, nephrogenous cyclic AMP excretion, and tumor burden were assessed in nine patients with gynecologic neoplasms and hypercalcemia. Gynecologic neoplasms were responsible for hypercalcemia in seven of 34 (20.5%) consecutive patients with malignancy-associated hypercalcemia. The tumor burden in each patient was large. Three of four endometrial carcinomas contained squamous elements. All patients displayed biochemical evidence of nonparathyroid humorally mediated hypercalcemia (bone resorption). Treatment of hypercalcemia did not appear to diminish production of the humoral calcemic factor but eradication of tumor eliminated biochemical evidence of the humoral syndrome. It can be concluded that (1) gynecologic neoplasms are a frequent cause of malignancy-associated hypercalcemia, (2) humoral mechanisms appeared to be responsible for the hypercalcemia in 100% of the patients in this series, (3) squamous features occur with unexpected frequency in hypercalcemic endometrial carcinoma, (4) the presence of hypercalcemia connotes a large tumor burden, and (5) treatment directed at the neoplasm (but not treatment directed at hypercalcemia) may eliminate evidence of ectopic calcemic hormone production. (Author abstract) (30 Refs)

15. THYROTROPIN BINDING AND ADENYLATE CYCLASE ACTIVATION IN NORMAL AND NEOPLASTIC HUMAN THYROID TISSUE: LACK OF EFFECT OF THYROGLOBULIN.

Clark OH, Gerend PL, Nissenson RA
Surgical Service (112), Veterans Admin. Medical Center, 4150 Clement St., San Francisco, CA, 94121
J Clin Endocrinol Metab; 54(6):1157-1163 1982

The effects of bovine TSH (bTSH) and bovine (bTg) and human thyroglobulin (hTg) on the binding of [125I]bTSH and the activation of adenylate cyclase (AC) were studied in both normal and neoplastic human thyroid homogenates in vitro using adenylate cyclase conditions. [125I]bTSH bound specifically and with high affinity to a particulate fraction from normal thyroid and from benign and malignant thyroid neoplasms; this binding was inhibited by unlabeled bTSH (approx Kd = 10.0 mU/ml for normal tissue; Kd = 2.5 mU/ml for thyroid neoplasms). Half-maximal activation of AC was obtained at a TSH concentration of approx 2.5 mU/ml for both normal and neoplastic thyroid tissue, indicating that the high affinity TSH receptors are coupled to AC, at least in the neoplastic thyroid tissue. bTg and hTg did not inhibit [125I]bTSH binding to high affinity TSH receptors in either normal or neoplastic thyroid tissue. bTSH increased AC activity up to 25-fold in neoplastic thyroid tissue and up to 4-fold in normal thyroid tissue, whereas at concentrations up to 1 mg/ml, bTg had no effect. The current study demonstrates that bTSH binds to specific, high affinity receptors and stimulates AC activity in both normal and neoplastic thyroid tissue. bTg and hTg, under the conditions used in this experiment, do not influence TSH binding to its high affinity receptor in these tissues. Since Tg does not influence high affinity TSH binding or AC activity in a particulate fraction rich in plasma membranes from normal or neoplastic thyroid tissue, it is unlikely to have a modulating role on TSH action at the thyroid plasma membrane. (Author abstract) (26 Refs)

16. DIFFERENCES IN THE HORMONAL STIMULATION OF NORMAL AND LEUKEMIC HUMAN PERIPHERAL LEUKOCYTES (MEETING ABSTRACT)

Paletta E, Schwarzmeier JD
First Medical Department, University of Vienna, Vienna, Austria
12th Workshop on Leukocyte Cultures, February 14-17, 1982, Vienna, Austria Wierner Medizinische Akademie; :A69 1982

We have measured the density of beta-adrenoceptors and the stimulation of adenylate cyclase (AC) by the beta-adrenergic agonist isoproterenol (INE), prostaglandin E1 (PGE1) and histamine (HIS) in normal as well as leukemic leukocytes. The number of beta-adrenoceptors on intact cells was determined using the beta-adrenergic antagonist 125I-iodohydroxybenzylpindolol (IHYP) as radioligand. One single class of high affinity binding sites for IHYP was identified on normal lymphocytes, monocytes and polymorphonuclear leukocytes (PMN). Maximal binding of IHYP, ie the quantity of beta-adrenoceptors present did not differ significantly among normal leukocytes. Fractionation of lymphocytes into B- and T-cells revealed significantly higher beta-receptor numbers on B than on T cells. Among leukemic cells tested, blast cells from AMOL (acute monocytic leukemia) and AMMOL (acute myelomonocytic leukemia) patients usually exhibited much higher beta-receptor densities than those from AML (acute myelocytic leukemia), and beta-receptor numbers were lowest in CLL (chronic lymphocytic leukemia) and ALL (acute lymphocytic leukemia). AC activity measured as cyclic adenosine 3', 5' - monophosphate (cAMP) produced per mg protein per min was studied in cell membrane preparations. Despite the fact that beta-receptors numbers were comparable in lymphocytes, monocytes and PMN, the latter showed a much weaker response to INE, but also to PGE1 and HIS. Results obtained using GTP and NaF as positive effectors of AC indicate that the poor hormonal responsiveness of PMN might be due to an impaired coupling between hormone receptors and catalytic unit of AC. Normal B lymphocytes showed a marked increase in cAMP production upon stimulation with INE, whereas T cells failed to respond. This relates to the different numbers of receptors identified on the two cell types. Among leukemic cells, the effectiveness of INE to stimulate AC corresponded to the beta-receptor numbers found to be present. AC reactivity to PGE1 and HIS followed the pattern of response to INE. (no Refs)

CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS

17. LYMPHOCYTE ADENOSINE RECEPTORS COUPLED TO ADENYLATE CYCLASE PROPERTIES AND [illegible] OCCURRENCE IN VARIOUS LYMP[illegible] SUBCLASSES.

Bonnafous JC, Dornand J, Favero J, Mani JC
Laboratoire de Biochimie des Membranes, ER CNRS 228 ENSCM, 8 rue de l'Ecole Normale, F-34075 Montpellier, France
J Recept Res; 2(4):347-366 1982

Lymphocyte adenosine receptors which are coupled to adenylate cyclase were characterized and their presence in various lymphocyte subclasses and lymphoid cell lines and their possible involvement in specific immunological functions of these cells were investigated. The stimulation of thymocyte adenylate cyclase by 5'-N-ethylcarboxamide adenosine was biphasic, while the responses to 2-chloro adenosine and N6-(L-2-phenylisopropyl) adenosine were not, which suggests the existence of Ra subtypes of adenosine receptors. (24 Refs)

18. TURN ON AND TURN OFF REACTIONS OF BETA-ADRENERGIC-SENSITIVE ADENYLATE CYCLASE IN CONTROL AND DESENSITIZED C6 GLIOMA CELLS.

Homburger V, Lucas M, Bockaert J

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FEBS Lett; 141(2):245-250 1982

The adenylate cyclase (AC) rate activation constant (ka) and deactivation constant (koff) were measured in a desensitized system in order to elucidate the molecular mechanisms for desensitization of C6 glioma cells by a beta-adrenergic agonist. Results suggested that the collision coupling model adequately describes the molecular events which occur during AC activation. The increase in the coupling constant occurring during desensitization appears to result from a decrease in ka and a rise in koff. (14 Refs)

19. EFFECT OF IONIZING RADIATION OF LYMPHOCYTE MEMBRANES. PART OF A COORDINATED PROGRAMME ON CELL MEMBRANE PROBES AS BIOLOGICAL INDICATORS IN RADIATION ACCIDENTS. FINAL REPORT FOR THE PERIOD 1 APRIL 1978-31 MARCH L981.

Ojeda SF

International Atomic Energy Agency, Vienna, Austria

Effect of Ionizing Radiation of Lymphocyte Membranes. Part of a Coordinated Programme on Cell Membrane Probes as Biological Indicators in Radiation Accidents. Final Report for the Period 1 April 1978-31 March 1981. Available from National Technical Information Service, Springfield, VA, as DE81700670:, 33 pp., 1981.

A study of the effects of low doses of irradiation on membrane receptors of lymphoid cells indicated that doses as low as 10 rad induced detectable changes in the antigen receptors of cell surfaces. Lymphoid cells from mice or rabbit lymph nodes, or circulating lymphocytes from human volunteers were irradiated and studied for their ability to bind antisera against the IgG membrane receptors. The isolated lymphoid cells were x-irradiated, and tested versus non-irradiated controls. They were incubated at 37 C for different times, and IgG-positive cells stained by the direct or indirect immunofluorescence technique. The percentage of IgG-positive cells was reduced by low-dose irradiation, and proved dose- and temperature-dependent. The disappearance phenomenon depends on the microtubular structure, metabolic energy, and levels of C-AMP. Only the reappearance phase is temperature-dependent and not affected by the drugs tested. The phenomenon is dose-rate dependent, showing greater sensitivity at lower dose/rates. Experiments using anti-Fc and anti-Fab portions of the surface molecule, appear to confirm a partial internalization of the surface molecule as cause (at least in rabbit cells). Similar experiments with human cells did not show a differential effect. Human T-cells and FC receptors of Mast cells did, however, indicate that these surface molecules are also modified by irradiation. (Author abstract)

20. INHIBITION OF ANTERIOR PITUITARY CYCLIC AMP PHOSPHODIESTERASE BY COLCHICINE AND VINBLASTINE.

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FEBS Lett; 141(1):25-28 1982

The inhibition of the anterior pituitary cyclic adenosine monophosphate phosphodiesterase (cAMP) by the microtubule inhibitors colchicine (COL) and vinblastine (VBL) was observed studying the possible role of the microtubules in the hormone secretion. Both alkaloids led to a significant increases in pituitary cAMP concentration as did theophylline and prostaglandin E2 that were included as positive controls. No effect of either COL (10(-3) M) or VBL (10(-4) M) upon pituitary adenylate cyclase activity was seen although prostaglandin E2 (10(-6) M) and sodium fluoride (10(-2) M) led to 2-and 4xX stimulations of the enzyme. In contrast, both alkaloids led to a concentration-dependent inhibition of the pituitary cAMP phosphodiesterase with about half-maximal effect being seen at 10(-3) M for both COL and VBL. The COL effect was seen in the presence or absence of divalent calcium (50 uM). Lumicolchicine, produced from colchicine by UV irradiation at 366 nanometers, was equipotent with COL in these studies. (16 Refs)

21. STIMULATION OF NEUROBLASTOMA ADENYLATE CYCLASE BY ARACHIDONIC ACID METABOLITES.

Howlett AC

Dept. Pharmacology, St. Louis Univ. Sch. Medicine, St. Louis, MO, 63104

Mol Pharmacol; 21(3):664-670 1982

Regulation of N18TG2 neuroblastoma adenylate cyclase (NAC) by prostaglandins (PG) metabolically derived from arachidonic acid was examined. Intact N18TG2 cells were examined for their ability to synthesize cyclic AMP (cAMP) in response to PGI1 and prostaglandins of the E type. PGI1 stimulated cAMP accumulation to the same maximal value as PGE1. An enzymatic derivative of PGI2, 6-keto-PGE1, stimulated cAMP accumulation to the same maximal value as PGE1. At 10 uM, PGE2 did not produce a maximal response. The 15-methyl analogue of PGE1 appeared to be more efficacious than PGE1, possibly because latter is not subject to the same metabolic pathways as other PG, although no evidence exists that N18TG2 cells have 15-OH-dehydrogenase activity. A similar pattern of response to PG was observed when NAC activity was examined in membranes prepared from N18TG2 cells. At 10 uM, maximal activity was achieved by PGE1, 15-methyl-PGE1, PGI2, and 6-keto-PGE1. This represents a stimulation of greater than 6-fold over that with guanosine triphosphate (GTP) alone. A pharmacological characterization of the arachidonic acid derivatives compared with PGE1 is shown. PGI2 was one order of magnitude more potent than PGE1 in simulating NAC activity. The receptors characterized in the study exhibit properties typical of receptors for other hormones that stimulate NAC. Maximally activating prostanoid agonists augment the response to GTP and guanosine 5'-(beta, gamma-imino)-triphosphate. This is consistent with a mechanism of more rapid occupancy of the GTP site by a nonhydrolyzable analogue. The prostanoid agonists reduce the requirement for free divalent cation. (31 Refs)

22. EFFECTS OF WR 2721 ON CYCLIC NUCLEOTIDE LEVELS AND LYSOSOMAL ENZYME ACTIVITIES.

Trocha PJ, Catravas GN
Biochemistry Dept., Armed Forces Radiobiology Res. Inst., Bethesda, MD, 20814
Int J Radiat Oncol Biol Phys; 8(3/4):543-546 1982

Effects of WR 2721 administered ip to Sprague-Dawley rats 30-40 min prior to exposure to 1,000 rad 60Co on cyclic nucleotide levels and lysosomal enzyme activities were studied. WR 2721 caused changes in cyclic nucleotide levels and beta-glucuronidase activities in spleen and liver and the changes were greater in the spleen than liver. It is suggested that WR 2721 imparts its radioprotective effect to mammalian systems by altering cell populations in radiosensitive tissues (spleen), resulting in modifications of cyclic nucleotide levels and lysosomal activities in irradiated and unirradiated animals. (7 Refs)

23. COVALENT CROSS-LINKING OF A PHOTOACTIVE DERIVATIVE OF CALCITONIN TO HUMAN BREAST CANCER CELL RECEPTORS.

Moseley JM, Findlay DM, Martin TJ, Gorman JJ
Dept. Medicine, Univ. Melbourne, Repatriation General Hosp., Heidelberg, Victoria, 3077, Australia
J Biol Chem; 257(10):5846-5851 1982

A photoaffinity derivative of salmon calcitonin has been produced by transglutaminase-mediated incorporation of N-(beta-aminoethyl)-4-azido-2-nitroaniline into the hormone. The derivative, purified by high pressure liquid chromatography, retained the abilities to bind to cultured T47D breast cancer cells and to stimulate adenylate cyclase in these cells. In both these respects it was equipotent with synthetic salmon calcitonin. Photolysis of the 125I-labeled photoactive salmon calcitonin derivative bound to T47D cells was accompanied by specific labeling to only one component ($Mr = 85,000$) as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Labeling was observed only upon photolysis and was inhibited by unlabeled synthetic salmon calcitonin but not by the inactive calcitonin analogue, 8-glycine human calcitonin. Reduction did not alter the apparent mol wt of the calcitonin receptor complex. No macromolecular forms of calcitonin were produced by photolysis in the absence of T47D cells. (Author abstract) (29 Refs)

CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES

24. URINARY CYCLIC NUCLEOTIDES AND THE CYTOPATHOLOGY OF HUMAN UTERINE CERVICAL DYSPLASIAS.

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Cancer Res; 42(7):2938-2943 1982

Blinded urinary assays for cyclic guanosine 3':5'-monophosphate (cGMP) and cyclic adenosine 3':5'-monophosphate (cAMP) were performed on 49 subjects with documented abnormal cervical cytology and 21 control subjects with normal cytology. A significant difference in the mean cGMP:cAMP ratios between the case and control groups was found. A significantly greater proportion of women with cytological abnormalities had a cGMP:cAMP ratio above the 0.2 level (p less than 0.001). Cases treated surgically for severe dysplasia or carcinoma in situ of the cervix revealed a significant postsurgical fall in the cGMP:cAMP ratios (p less than 0.025). The possibility of utilizing urinary ratios of cyclic nucleotides as an objective index in the detection, monitoring of progression, and therapy of preneoplastic cervical lesions is discussed. (Author abstract) (27 Refs)

25. GUANYLATE CYCLASE ACTIVITY IN HUMAN LEUKEMIC AND NORMAL LYMPHOCYTES. ENZYME INHIBITION AND CYTOTOXICITY OF PLANT EXTRACTS.

Takemoto DJ, Dunford C, Vaughn D, Kramer KJ, Smith A, Powell RG
Dept. Biochemistry, Kansas State Univ., Manhattan, KS, 66506
Enzyme; 27(3):179-188 1982

Cyclic GMP is thought to be involved in lymphocytic cell proliferation and leukemogenesis. In general, the nucleotide is elevated in leukemic vs normal lymphocytes and changes have been reported to occur during remission and relapse of this disease. Although the cA/cG ratios are higher for normal lymphocytes the basal guanylate cyclase (EC 4.6.1.2) activities do not correlate with altered cyclic GMP levels. The crude guanylate cyclases display classical Michaelis-Menten kinetics with Kms for Mn-GTP of 463 umol/l and 20-90 umol/l for normal and leukemic lymphocytes, respectively. An extract from the bitter melon (momordica charantia) preferentially inhibits the soluble guanylate cyclase from leukemic lymphocytes. This inhibition correlates with its preferential cytotoxic effects for these same cells. Analyses of nine other cytotoxic plants extracts revealed that only an extract from the Lawson's cypress, Chamaecyparis lawsonianna, exhibits comparable cytotoxicity and guanylate cyclase inhibition levels. (Author abstract) (29 Refs)

PROTEIN KINASE STUDIES

26. CYCLIC ADENOSINE 3':5'-MONOPHOSPHATE-DEPENDENT PROTEIN PHOSPHORYLATION AND THE CONTROL OF LEUKEMIA L1210 CELL GROWTH.

Mednieks MI, Jungmann RA, DeWys WD
Lab. Biological Structure, Natl. Inst. Dental Res., Building 30, Room 211, Bethesda, MD, 20205
Cancer Res; 42(7):2742-2747 1982

Synergistic increases in the survival of mice bearing an L1210 leukemia tumor have been demonstrated previously after treatment with 1,3-bis(2-chloroethyl)-1-nitrosourea together with theophylline over those treated with either agent alone. These results imply that manipulation of cyclic adenosine 3':5'-monophosphate (cyclic AMP) levels in L1210 cells may result in alteration of sensitivity to chemotherapy and alterations in tumor growth. In the present study, we have shown that in vivo treatment of L1210 cells with theophylline results in changes in intracellular cyclic AMP-dependent protein kinase activity levels as well as in an apparent redistribution of both the nuclear and cytoplasmic isozymes. Biochemical events in the tumor cells immediately after administration of theophylline in vivo or a cyclic AMP analog (8-parachlorophenylthio cyclic adenosine 3':5'-monophosphate) in vitro were independent of the presence of 1,3-bis(2-chloroethyl)-1-nitrosourea. The changes apparently involve signal transduction via the adenylate cyclase system and manifest as: (a) increased sensitivity of cyclic AMP-dependent protein kinase to activation by cyclic AMP after treatment of L1210 cells with theophylline; (b) decrease in endogenous nuclear protein phosphorylation sites; and (c) protein kinase isozyme redistribution between nuclear and extranuclear compartments, ie, a relative increase of the Type I isozyme activity in the nuclear and of the Type II isozyme activity in the 900 x g supernatant fractions after treatment of the mice of theophylline. The relative activity increases are accompanied by relative decrease of Type II activity from the nucleus and Type I isozyme activity from the 900 x g extranuclear supernatant fraction. These events appear temporally related to changes in nuclear RNA metabolism as evidenced by altered kinetics of RNA precursor uptake and incorporation into tumor cell RNA after treatment. These results imply that the cyclic AMP-dependent phosphorylative modification of intracellular proteins may play a regulatory role in tumor cell growth and in theophylline-mediated tumor regression. (Author abstract) (42 Refs)

27. INTERFERON INDUCTION OF POLYAMINE-DEPENDENT PROTEIN KINASE ACTIVITY IN EHRLICH ASCITES TUMOR CELLS.

Sekar V, Atmar VJ, Krim M, Kuehn GD
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Biochem Biophys Res Commun; 106(2):305-311 1982

The finding of an interferon-induced protein kinase activity in Ehrlich ascites tumor cells which is activated by the polyamines spermidine and spermine is reported. The cytoplasmic fraction of interferon-treated cells demonstrated and enhanced level of protein kinase activity compared to controls, and the activity was enhanced 2.8x by the polyamines or by poly(I).poly(C). Simultaneous additions of the polyamines and double stranded RNA produced a 4x stimulation. The nuclear fraction of interferon-induced cells also exhibited a marked enhancement of protein kinase activity, with a pattern similar to that observed in the cytoplasmic fraction. Interferon elicited a 33x induction of polyamine-dependent protein kinase activity in the nucleus. Combined additions of the polyamines and double stranded RNA evoked no significantly different response than polyamines alone, suggesting that a single protein kinase may be activated both by a spermidine/spermine mixture and by double stranded RNA. Partial purification of cell extracts on poly(I).poly(C)-agarose or by fractionation on phosphocellulose columns supported this suggestion. (16 Refs)

28. PHOSPHORYLATION OF TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE IN LEUKEMIC CELLS.

Elias L, Longmire J, Wood A, Ratliff R
Dept. Medicine, Univ. New Mexico Sch. Medicine, Albuquerque, NM, 87131
Biochem Biophys Res Commun; 106(2):458-465 1982

Because of the specific involvement of protein phosphorylation in malignant transformation, the possibility that terminal deoxynucleotidyl transferase (TdT) might act as a physiological substrate for leukemic cellular protein kinase (PK) was investigated. NALM-6 (TdT-positive human lymphoblastic leukemia) cells were labeled with 32-PO4 and TdT was recovered from the lysate by phosphocellulose chromatography followed by immunoprecipitation. Electrophoresis revealed a major labeled band of mol wt 59,000 corresponding to the high mol wt form of TdT characteristic of human leukemic cells. The phosphoprotein was recovered and subjected to phosphoroamino-acid analysis, which disclosed 85% phosphoserine and 15% phosphothreonine. Purified calf thymus TdT was phosphorylated in vitro by crude and partially purified human leukemic cell PK. TdT phosphorylation by leukemic cell extracts and semi-purified PK was not stimulated by cAMP. Phosphorylation of TdT was shown to increase activity significantly, particularly in the presence of high concentrations of riboATP, as occur intracellularly. These results suggest a mechanistic link between two enzymatic systems, each of which is of largely unknown function but both of which are prominently associated with leukemia. TdT phosphorylation may be part of a coordinated general pathway pertaining to control of gene transcription and protein synthesis which is appropriately activated in proliferating normal and malignantly transformed cells. (20 Refs)

29. DIRECT CYTOCHEMICAL LOCALIZATION OF CATALYTIC SUBUNITS DISSOCIATED FROM CAMP-DEPENDENT PROTEIN KINASE IN REUBER H-35 HEPATOMA CELLS. I. DEVELOPMENT AND VALIDATION OF FLUORESCINATED INHIBITOR.

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J Cell Biol; 93(3):719-726 1982

A specific and sensitive procedure has been developed that reliably localizes intracellular sites of free catalytic unit (C) dissociated from cAMP-dependent protein kinase. The method is based on a FITC conjugate (F:PKI) of affinity column-purified heat-stable protein inhibitor (PKI) of free C. The fidelity of this cytochemical probe was determined using cultures of Reuber H-35 hepatoma cells that had been stimulated for 2 hr with 0.1 mM DBcAMP, or with diluent, then fixed with anhydrous acetone at-30 C. In these preparations the F:PKI probe complexed with free C in cytoplasm, nucleolus, and, to a minor extent, in nucleoplasm. Binding of the F:PKI molecule to free C was competitively diminished by arginine analogues, guanidinium HCl and polyarginine, each used over a 2-log dose range. When the inhibitor's arginine residues were blocked by reaction with cyclohexanedione it no longer inhibited phosphotransferase activity of free C, and when fluorescinated it failed to localize C in stimulated cells. Similarly, when F:PKI was preabsorbed with excess pure C it no longer functioned as a cytochemical stain. Affinity column-purified antibody to free C also reduced significantly the ability of F:PKI to complex with C in cell cultures stimulated with 0.1 mM DBcAMP. One microgram of antibody reduced by approx 10% the binding of F:PKI to all cell compartments while 5 ug of antibody diminished binding by greater than 50%. Together, these results indicate that the F:PKI binds specifically, perhaps exclusively, to the catalytic units of cAMP-dependent protein kinase. The cytochemical procedure, unlike its biochemical counterparts, is able to locate the dissociation of cAMP-dependent protein kinase in individual cells of functionally or histologically complex cultures. Also, it reveals variations in the time- and dose-dependent activation of the kinase amongst clonal cells stimulated with cyclic nucleotide analogues or hormones. (Author abstract) (34 Refs)

30. DIRECT CYTOCHEMICAL LOCALIZATION OF CATALYTIC SUBUNITS DISSOCIATED FROM CAMP-DEPENDENT PROTEIN KINASE IN REUBER H-35 HEPATOMA CELLS. II. TEMPORAL AND SPATIAL KINETICS.

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J Cell Biol; 93(3):727-734 1982

The activation of cyclic AMP-dependent protein kinase results in the release of the catalytic subunit which as the free enzyme possesses phosphotransferase activity for a variety of specific protein substrates. Using a cytofluorometric technique we monitored the appearance of free catalytic subunit in Reuber H35 hepatoma cells in culture after incubation with N(6)-1'-O-dibutyryl-cyclic AMP (DBcAMP), 8-bromoadenosine-3':5'-cyclic monophosphate (8-BrcAMP), and glucagon. The cytochemical method employs the heat-stable inhibitor of the free catalytic subunit which has been conjugated to fluorescein isothiocyanate (F:PKI). Here we studied the temporal and spatial kinetics of the free catalytic subunit following activation of cAMP-dependent protein kinase by increasing concentrations of DBcAMP,8-BrcAMP, and glucagon. Under similar conditions protein kinase activation was also assessed biochemically in H35 cell supernatants by assaying the protein kinase activity ratio. Incubation of the hepatoma cells with DBcAMP led to an increase in the activity ratio. During the same period using the F:PKI probe, a significant increase in cytoplasmic and nucleolar fluorescence indicative of the release of the free catalytic subunit was coincidentally observed. Discernible nucleoplasmic fluorescence did not occur until 1 hr after the addition of DBcAMP. Cultures incubated with 8-BrcAMP exhibited a more rapid activation of the protein kinase measured cytochemically. Cultures incubated with 8-BrcAMP had significantly increased cytoplasmic and nucleolar fluorescence compared to unstimulated cells. The results reported herein suggest that both cyclic nucleotide analogues and a polypeptide hormone lead to the activation of cAMP-dependent protein kinase in similar intracellular compartments in Reuber H35 hepatoma cells. The appearance of protein kinase catalytic subunit in the nucleolus also serves to support earlier reports which suggest a biological role for this enzyme in the nucleus. (33 Refs)

31. EFFECT OF 3-METHYLCHOLANTHRENE ON RNA POLYMERASE AND PROTEIN KINASE ACTIVITIES OF TRANSCRIPTIONALLY ACTIVE CHROMATIN SUBFRACTION IN RAT LIVER.

Szeberenyi J, Kleeberg U, Tigyi A

Dept. Biology, Univ. Medical Sch., Pecs, Hungary

Experientia; 38(6):687-689 1982

The activities of RNA polymerases and nuclear protein kinases were determined in subfractions of rat liver nuclei after a single dose of the polycyclic aromatic hydrocarbon 3-methylcholanthrene (3-MC). Both enzyme activities as well as the in vivo phosphorylation of chromatin proteins were enhanced by 3-MC in the transcriptionally active subfraction of chromatin. (Author abstract) (18 Refs)

32. CARBOXY TERMINUS OF POLYOMA MIDDLE-SIZED TUMOR ANTIGEN IS REQUIRED FOR ATTACHMENT TO MEMBRANES, ASSOCIATED PROTEIN KINASE ACTIVITIES, AND CELL TRANSFORMATION.

Carmichael GG, Schaffhausen BS, Dorsky DI, Oliver DB, Benjamin TL

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Proc Natl Acad Sci USA; 79(11):3579-3583 1982

We have constructed a transformation-defective polyoma virus mutant (Py 1387-T) that directs the synthesis of a normal small tumor antigen, a functional large tumor antigen, and a truncated (51,000-dalton) middle-sized tumor (mT) antigen that lacks 37 amino acids at its COOH terminus. The shortened mT polypeptide is missing the hydrophobic 'tail' thought to be responsible for the anchorage of this protein into the plasma membrane and is in fact in cytosol fractions. This truncated mT polypeptide is inactive in an in vitro protein kinase assay and is altered in its phosphorylation in vivo. Mutant 1387-T differs from wild-type virus in having a T-A base pair instead of a C-G base pair at nucleotide position 1387. This change was introduced into viral DNA by using a synthetic undecanucleotide as a specific mutagen. Wild-type polyoma DNA was rendered single stranded by molecular cloning into coliphage M13. The oligonucleotide, which hybridizes with a mismatch at the site to be altered, was used to prime the synthesis of double-stranded closed circular DNA. Progeny recombinant phages were screened by DNA sequence analysis for the desired base change. The polyoma mutant was reconstructed from recombinant phage replicative form DNA molecules containing the mutation. (Author abstract) (45 Refs)

33. ALTERATION OF THE REGULATORY SUBUNIT OF TYPE 1 CAMP-DEPENDENT PROTEIN KINASE IN MUTANT Y1 ADRENAL CELLS RESISTANT TO 8-BROMOADENOSINE 3':5'-MONOPHOSPHATE.

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J Biol Chem; 257(10):5877-5883 1982

The biochemical basis for cellular resistance to ACTH and cAMP was examined in a family of mutant clones (Kin) derived from the Y1 adrenocortical tumor cell line. Extracts from the Kin mutants bound [3H]cAMP poorly when compared with extracts from parental Y1 cells. The degree of impairment in cAMP-binding activity among Kin mutants corresponded with the degree of impairment in cAMP-dependent protein kinase activity demonstrated previously. Impaired cAMP-binding activity in several Kin mutants also was observed with the photoreactive probe, 8-azido-[32P]cAMP, and appeared to result from alterations in the Type 1 cAMP-dependent protein kinase. The holoenzymes from the parent Y1 and two mutant Kin clones were separated into catalytic and Type 1 regulatory (RI) subunits. Homologous and heterologous holoenzymes were formed from the isolated subunits and assayed for cAMP-dependent protein kinase activity. Analysis of the reconstituted holoenzymes indicated that the RI subunit was responsible for the altered cAMP-dependent protein kinase activity observed in the two Kin mutants. Y1 and mutant Kin clones were labeled with L-[35S]methionine and the RI subunits were immunoprecipitated. When analyzed on isoelectric focusing gels, the immunoprecipitates from the two mutants contained an acidic variant form of RI. This variant species of RI appears to be related to the altered phenotype of the Kin mutants, and may be responsible for the observed impairments in cAMP-dependent protein kinase activity and cAMP-binding activity. (Author abstract) (34 Refs)

34. ISOLATION OF TRANSFORMATION-DEFECTIVE HOST-RANGE MUTANTS OF POLYOMA VIRUS ON NORMAL MOUSE CELLS.

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Virology; 119(2):310-316 1982

Host-range mutants of polyoma virus have been isolated utilizing primary mouse embryo fibroblasts as the permissive host and cells of an established 3T3 line as the nonpermissive host. Five out of five such 'mouse embryo-dependent' (MED) mutants have totally lost transforming ability. Their transformation defects can be complemented by ts-a mutants but not by hr-t mutants. Restriction enzyme digestion shows deletions of 150-200 base pairs in the proximal early region of the viral DNAs (HpaII fragment 4, 79-92 map units). None of the MED mutants produce middle or small tumor antigen species detectable by metabolic labeling and immune precipitation, and none show the middle T-antigen-associated protein kinase activity in vitro. MED mutants are thus indistinguishable from hr-t mutants previously isolated ising polyoma-transformed 3T3 cells as the permissive host. The isolation of nontransforming host-range mutants in the absence of direct polyoma gene complementation confirms a specific prediction of the hypothesis of indirect action by the hr-t gene, according to which the inability to transform must arise concomitantly with the inability to induce a permissive state for virus growth. (Author abstract) (28 Refs)

35. CHARACTERIZATION OF THE AVIAN SARCOMA VIRUS TRANSFORMING GENE PRODUCTS (MEETING ABSTRACT)

Erikson RL, Gilmer TM, Erikson E, Arrigo AP, Foulkes JG, Granot Y

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Conference on Cell Proliferation, Cancer and Cancer Therapy, February 17-19, 1982, New York, New York New York Academy of Sciences; :A24 1982

The Rous sarcoma virus transforming gene product (pp60src) and its normal cell homologue both appear to be protein kinases with the capacity to phosphorylate tryosine residues in various protein substrates. Investigations in several laboratories have shown that at least three other classes of avian sarcoma viruses exist each with a unique transforming gene encoding transforming gene products with associated tyrosine-specific phosphotransferase activity. Some of these transforming gene products have been purified extensively and the nature of their associated enzymatic activities will be discussed. Studies on radiolabeled phosphoproteins in infected cells show that dephosphorylation of phosphotyrosine can occur very rapidly, thus suggesting the existence of a phosphotyrosine phosphatase(s). Progress in the purification of the relevant enzymes and their activities, particularly toward pp60src, will be discussed. (no Refs)

36. ORIGIN, STRUCTURE, AND FUNCTION OF THE TRANSFORMING REGION ON THE ABELSON MURINE LEUKEMIA VIRUS (MEETING ABSTRACT)

Baltimore D, Wang J, Goff S, Queen C

Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA

Thirty-fifth Annual Symposium on Fundamental Cancer Research, March 2-5, 1982, Houston, Texas. The University of Texas M. D. Anderson Hospital and Tumor Institute 38 pp., 1982.

The transforming region, V-abl, of the Abelson murine leukemia virus (A-MuLV) originates in a normal gene of the mouse, C-abl. C-abl represents a number of small regions of DNA that are homologous to V-abl separated by long stretches of DNA that are not. Our interpretation of this organization is that V-abl represents a cDNA copy of the exons that compose the C-abl gene and that the large nonhomologous regions represent introns that have been excised. V-abl joins the MuLV sequence in the A-MuLV genome at a precise point where there is a four-base homology between the Moloney murine leukemia virus (M-MuLV) sequence and the C-abl sequence. The joining position appears to be in the center of an exon of the C-abl gene. At the 3' end, the V-abl/M-MuLV joint is again precise, but the C-abl sequence has not been completed in that region. The organization of the transcripts of the C-abl gene indicates that the C-abl mRNA (of which there are two) has 400 bases 5' of the region homologous to V-abl and 2 to 3 kb 3' of the homologous region. All these structural facts can be reconciled by assuming that the A-MuLV genome arose through the integration of a M-MuLV genome upstream from the C-abl gene, followed by a deletion that brought the M-MuLV sequences in apposition to a C-abl exon. Transcription of this deleted gene from the M-MuLV promoter would then generate an RNA transcript with an M-MuLV 5' end. The final A-MuLV genome presumably arose by a secondary recombination at the reverse transcription level, according to the model of Goldfarb and Weinberg. In the context of the A-MuLV genome, the V-abl sequences encode a tyrosine-specific protein kinase activity. By expressing the V-abl region in Escherichia coli, the kinase activity has been shown to be intrinsic to the V-abl protein because in Escherichia coli the protein is phosphorylated on a tryosine residue. (1 Ref)

37. THREE CLASSES OF AVIAN SARCOMA VIRUSES (MEETING ABSTRACT)

Neil JC, Ghysdael J, Vogt PK

No affiliation given

10th International Symposium for Comparative Research on Leukemia and Related Diseases, August 31-September 4, 1981, Los Angeles, California. International Association for Comparative Research on Leukemia and Related Diseases 187 pp., 1981.

Three classes of avian sarcoma viruses have been defined in recent studies on the transformation-specific nucleic acid and protein sequences of these agents. The first class consists of Rous sarcoma virus and its relatives, the second contains PRCII sarcoma virus and variants, and Fujinami sarcoma virus, while the third class comprises Y73 virus and recently characterized isolate, Esh sarcoma virus (ESV). Class I viruses encode pp60src, while class II and III viruses code for fusion proteins in which helper virus gag sequences are lined to a transformation-specific portion. All three classes of virus have tyrosine-specific protein kinase activity associated with the transformation-specific proteins. Our present studies are aimed at the identification of normal cellular homologues of the class II and III viral proteins. This information would assist in the search for common features of transformation by the three classes of sarcoma viruses. (4 Refs)

38. TEMPERATURE-SENSITIVE MUTANTS OF AVIAN SARCOMA VIRUS PRCII: COORDINATE DEFECTS IN THE MAINTENANCE OF TRANSFORMATION AND IN THE STRUCTURE AND FUNCTION OF THE TRANSFORMATION-SPECIFIC PROTEIN P105 (MEETING ABSTRACT)

Vogt PK, Hirano A
No affiliation given
10th International Symposium for Comparative Research on Leukemia and Related Diseases, August 31-September 4, 1981, Los Angeles, California. International Association for Comparative Research on Leukemia and Related Diseases 187 pp., 1981.

PRCII belongs to the new category of avian sarcoma viruses which, similar to avian acute leukemia viruses, code for a gag-linked polyprotein as their transformation-specific product. The gag sequences of this molecule contain p19 and at least part of p27, while the transformation-specific portion proper is derived from a cellular genetic substitution that shows homology to a similar genetic element in the genome of Fujinami sarcoma virus and has been termed fps. Circumstantial evidence suggests a role for gag-linked proteins in the maintenance of oncogenic transformation. Genetic data favoring such a role are, however, still incomplete. We have isolated mutants of PRCII which are temperature-sensitive (ts) in the maintenance of transformation, Chick embryo fibroblasts transformed by such mutants at 36 degrees acquire normal phenotype when the temperature is shifted to 41.5 degrees and revert to the transformed state upon down-shift to 36 degrees. The lesion in the PRCII transformation mutants cannot be complemented by wild type helper virus and therefore appears unrelated to the function of the replicative retrovirus genes gag, pol or env: Transforming virus rescued from ts mutant-infected nonproducing clones with wild type helper viruses remain ts in transformation. However, the ts transformation is correlated with immunological detectability of the transformation-specific, gag-linked polyprotein p105 and its associated protein kinase. At the nonpermissive temperature amounts of p105 precipitable with anti-gag antibody and kinase activity in the precipitates are greatly reduced. In shift experiments p105 associated kinase activities parallel the emergence or disappearance of the transformed phenotype. (no Refs)

39. DECREASED STEROID PRODUCTION IN ACTH-PRETREATED Y-1 ADRENAL TUMOR CELLS RELATED TO CAMP-DEPENDENT PROTEIN KINASE ACTIVATION (MEETING ABSTRACT)

Murray SA, Byus CV, Fletcher WH
Department of Biochemistry, University of California, Riverside, CA
Anat Rec; 202(3):134A 1982

Y-1 mouse adrenal cortical tumor cells increase the production of steroids and cAMP while decreasing DNA synthesis in response to ACTH. ACTH pretreated cells become resistant to the effect of subsequent ACTH on steroid production, cAMP synthesis, and the inhibitory effect on DNA synthesis. DB cAMP pretreatment of the Y-1 cells results in a resistance of cells to respond to ACTH or further dB or 8BrcAMP in terms of steroid production. Employing a fluorescinated conjugate of the heat stable inhibitor protein as a specific and sensitive cytochemical probe we have examined the temporal and spatial kinetics of catalytic subunit (C) dissociated from the cAMP-dependent protein kinase holoenzyme at a single cell level of resolution. When Y-1 adrenal cells were stimulated with ACTH (0.5-50 milliunits/ml) for 30 second to 1 hour cAMP-dependent protein kinase was dissociated in a dose and time dependent manner. Free C appeared first in the cytoplasm and in the nucleolus and with time nuclear fluorescence was observed. Y-1 adrenal cells pretreated with ACTH or DB cAMP had a decreased activation of the kinase following subsequent ACTH treatment. (no Refs)

40. IDENTIFICATION OF TWO INHIBITORS OF HEPATOMA POLYAMINE RESPONSIVE PROTEIN KINASE SYSTEM (MEETING ABSTRACT)

Morishita Y, Sahai A, Akogyeram C, Liederman L, Oka T, Criss WE
Cancer Center, Washington, DC, 20059
Med Pediatr Oncol; 10(1):102 1982

A polyamine responsive kinase (PPK) system was first identified and purified from the cytosol of a series of fast growing Morris hepatomas. The native enzyme was resolved into a catalylic component (MW 210,000) and several substrates or phosphate acceptor proteins (all less than MW 100,000). This report presents data that the hepatoma PPK systems also contains two dialyzable inhibitor molecules. The inhibitors were identified and purified through two different purification schemes: 1) DEAE (diethylaminoethyl) cellulose, Sephacryl S-300, Sephadex G-25, and phosphocellulose column chromatographies; 2) extensive dialysis using a 50,000 MW exclusion dialysis membrane, lypholyzation of dialyzing medium, and Sephadex G-50 column chromatography. Inhibitor I had a molecular weight of 500-600 and inhibited both the basal and polyamine stimulatable activities when the dialyzed enzyme was evaluated. Inhibitor II had molecular weight near 1,800 and also inhibited both basal and stimulatable activities when the dialyzed enzyme was evaluated. Both inhibitors were heat stable and appear to interact with the substrate binding site on the intact enzyme. They are bound to the enzyme in vivo, as the entire system, catalytic unit, substrates, and inhibitors copurify in the initial purification stages. (1 Ref)

OTHER RELATED STUDIES

41. INSULIN RELEASE AND PROTEIN PHOSPHORYLATION: POSSIBLE ROLE OF CALMODULIN.

Schubart UK, Erlichman J, Fleischer N
Dept. Medicine and Diabetes Res. and Training Center, Albert Einstein Coll. Medicine, Bronx, NY, 10461
Fed Proc; 41(7):2278-2282 1982

Both Ca++ and cyclic AMP (cAMP) are implicated in the regulation of insulin release in the pancreatic beta cell. In hamster insulinoma cells used in our laboratory to study the mechanism of insulin release, Ca++ and cAMP trigger secretion independently. Concomitant with stimulation of the secretory apparatus both cAMP and Ca++ promote phosphorylation of distinct insulinoma cell proteins. Calmodulin may be involved in the stimulation of insulin release and protein phosphorylation induced by Ca++ influx. The Ca++-dependent protein kinase of the insulinoma cell is activated by exogenous calmodulin and blocked by trifluoperazine, an inhibitor of calmodulin action. This drug also inhibits glucose-induced insulin release in pancreatic islets. In insulinoma cells trifluoperazine blocks Ca++ influx-mediated insulin release and protein phosphorylation with no effect on basal or cAMP-mediated secretion. Inhibition of Ca++ influx-mediated insulin release and protein phosphorylation occurs with nearly identical dose dependence. Inasmuch as trifluoperazine affects voltage-dependent Ca++ uptake in insulinoma cells, an involvement of calmodulin cannot be directly inferred. The evidence suggests that protein phosphorylation may be involved in the activation of the secretory apparatus by both cAMP and Ca++. It is proposed that stimulation of insulin release by cAMP and Ca++ is mediated by cAMP-dependent protein kinase and calmodulin-dependent protein kinase, respectively. (Author abstract) (43 Refs)

42. CALCIUM: CALMODULIN AND CANCER.

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Fed Proc; 41(7):2289-2291 1982

When several fast-growing Morris hepatoma tissue lines are compared with normal adult liver tissue, the following observations are made: calmodulin activity is increased in the cytoplasm and decreased in the membranes of the tumor cells. Total calcium is increased three- to fivefold in the tumors. Cyclic AMP phosphodiesterase activity is increased, whereas cyclic GMP phosphodiesterase activity is decreased. In addition, several of the fast-growing Morris hepatoma tissue lines have a new calcium-binding protein that is not observed in adult liver tissue. It is probable that the Ca++-calmodulin complex is very active in these rapidly growing tumors. (Author abstract) (21 Refs)

43. CALMODULIN, ACTIVATED CYCLIC NUCLEOTIDE PHOSPHODIESTERASE, MICROTUBULES, AND VINCA ALKALOIDS.

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Dept. Pharmacology, Howard Univ. Coll. Medicine, Washington, DC, 20059

Fed Proc; 41(7):2292-2299 1982

Calmodulin, 1.8 and 10.6 uM, inhibited the polymerization of bovine brain microtubules by 30 and 50%, respectively. Two 55,000-to 68,000-dalton calmodulin-binding proteins as well as calmodulin-dependent and -independent phosphodiesterases (PDE) were found associated with microtubule proteins. Among the antimicrotubule drugs, such as colchicine, podophyllotoxin, griseofulvin, and vinca alkaloids (vinblastine, desacetylvinblastine amide, and vincristine), the vinca alkaloids were selective inhibitors of calmodulin-activated PDE activity. This action of vinca alkaloids resides in the catharanthine moiety of vinblastine molecule. An alpha2 inhibitor, yohimbine, affects the microtubules, and a series of alpha-adrenoceptor blocking agents were examined for their effects on calmodulin-dependent PDE. The relative order of the potency is phenoxybenzamine = dibenamine greater than phentolamine greater than yohimbine greater than prazosin greater than tolazoline, and the first four drugs in this series were selective inhibitors of calmodulin action. Inasmuch as phenoxybenzamine and dibenamine are alkylating agents, the effects of antineoplastic alkylating agents on the calmodulin action were also examined. Busulphan, melphalan, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, and streptozotocin (up to 4 mM) were not selective inhibitors of calmodulin action. Maytanisine, a vinca alkaloid-type antimicrotubule agent as well as an alkylating agent, selectively inhibited the calmodulin with a potency similar to vincristine. In addition, phenoxybenzamine affected Ca++-dependent fluorescence induced by the interaction between calmodulin and hydrophobic fluorescent probes, whereas vinblastine was ineffective. However, the binding of vinblastine to calmodulin is calcium dependent. Studies such as these suggest the importance of physical and structural considerations in drugs binding to calmodulin was well as at least two different binding sites for drugs on calmodulin. (Author abstract) (52 Refs)

AUTHOR INDEX WITH CITATION NUMBERS

Akiyama M	2
Akogyeram C	40
Arrigo AP	35
Atmar VJ	27
Baltimore D	36
Barber R	8
Beavo JA	33
Benjamin TL	32
Benjamin TL	34
Berenblum I	6
Bertram BB	3
Bertram JS	3
Bockaert J	18
Bonnafous JC	17
Broadus AE	14
Byus CV	29
Byus CV	30
Byus CV	39
Carmichael GG	32
Catravas GN	22
Chou JY	4
Clark OH	15
Criss WE	40
Criss WE	42
DeWys WD	26
Doherty PJ	33
Dornand J	17
Dorsky DI	32
Dunford C	25
Duttagupta C	24
Elias L	28
Erikson E	35
Erikson RL	35
Erlichman J	41
Ewart RB	20
Favero J	17
Feunteun J	34
Findlay DM	23
Fleischer N	41
Fletcher WH	29
Fletcher WH	30
Fletcher WH	39
Forrest AP	10
Forrest AP	9
Foulkes JG	35
Friis RR	7
Gerend PL	15
Ghysdael J	37
Gilmer TM	35
Glossmann H	7
Goff S	36
Goka TJ	8
Gorman JJ	23
Granner DK	1
Granot Y	35
Hamprecht B	11
Hargrove JL	1
Hawkins RA	9
Hayashi Y	2
Hcumann R	11
Higashida H	2
Hirano A	38
Homburger V	18
Howard RF	7
Howlett AC	21
Janik P	3
Jungmann RA	26
Kakiuchi S	42
Katada T	13
Kather H	12
Kleeberg U	31
Kohorn EI	14
Kramer KJ	25
Krim M	27
Kuehn GD	27
Kuo CH	2
Liederman L	40
Lin T	5
Longmire J	28
Lucas M	18
Mani JC	17
Martin TJ	23
Mednieks MI	26
Miki N	2
Miller WR	10
Morishita Y	40
Moseley JM	23
Mumby MC	33
Murono EP	5
Murray SA	39
Neil JC	37
Nissenson RA	15
Ojeda SF	19
Oka T	40
Oliver DB	32
Paietta E	16
Palan PR	24
Powell RG	25
Queen C	36
Ratliff R	28
Reiser G	11
Romero R	14
Romney SL	24
Sahai A	40
Schaffhausen BS	32
Schimmer BP	33
Schroder F	12
Schubart UK	41
Schwartz PE	14
Schwarzmeier JD	16
Sekar V	27
Senbanjo R	9
Senbanjo RO	10
Sheppard JR	7
Slagle NS	24
Smith A	25
Stewart AF	14
Szeberenyi J	31
Takemoto DJ	25
Telford J	10
Telford J	9
Tigyi A	31
Trocha PJ	22
Tsao J	33
Ui M	13
Van Calker D	11
Vaughn D	25
Vogt PK	37
Vogt PK	38
Wang J	36
Watanabe K	43
Watson D	10
Watson D	9
West WL	43
Wood A	28

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In this issue:

- REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES 1-11
- REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES 12-13
- CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES 14-20
- CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS 21-22
- PROTEIN KINASE STUDIES 23-30
- OTHER RELATED STUDIES 31-34

REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES

1. MORPHOLOGICAL EVIDENCE FOR CYCLIC AMP-INDUCED REVERSE TRANSFORMATION IN VOLE CELLS INFECTED WITH AVIAN SARCOMA VIRUS.

Meek WD
Univ. Colorado Health Science Center, Denver, CO, 80262
Mol Cell Biol; 2(7):863-874 1982

Ultrastructural changes that result from dibutyryl cyclic AMP treatment were studied in vole cells. Scanning electron microscopy of the avian sarcoma virus-transformed cells showed the spherical cells covered with microvilli. Filopodia like those seen on mitotic normal cells radiated from the cell body. With fluorescent phalloidin, a rim of actin was noted below the cell membrane of the spherical cells. With an increasing concentration of reverse transformation (RT) agent, more cells became flattended and resembled the normal fibroblasts. Coating the substrate with polylysine did not affect the transformed cell shape when grown in normal medium, but the coated substrate increased the RT response. The ruffling that

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was common to normal cells occurred in some RT cells, but most cells had rigid, smooth borders. Transmission electron microscopy confirmed the results seen with antitubulin indirect immunofluorescence and the present results with fluorescent phalloidin and antiactin: long tubules ran parallel to the subplasmalemmal microfilament bundles, and intermediate filaments were parallel to the other microfibrils and prevalent in slender processes. Fibronectin was absent on the surface of the transformed vole cell. The long strands which extende from cell to cell in normal cultures were also absent. However, in the RT vole cell, short fibronectin fibrils appeared along the cell periphery. (47 Refs)

2. CYTOSTATIC AND REVERSE-TRANSFORMING THERAPIES OF CANCER. A BRIEF REVIEW AND FUTURE PROSPECTS.

McCarty MF
McNaughton Foundation, 1515 Madison Ave., San Diego, CA, 92116
Med Hypotheses; 8(6):589-612 1982

Cyclic AMP (cAMP) congeners and promoters, prostaglandin precursors, interferon, chalones, antineoplaston A, protease inhibitors, and polar solvents are reviewed. Efforts have been made to use congeners and promoters of cAMP to treat solid and ascitic animal tumors in vivo. Owing to its instability, cAMP is effective only if applied intratumoraly. The phosphodiesterase inhibitor papaverine induces reverse transformation of neuroblastoma in vitro, and was found to induce temporary remission in some clinical cases of neuroblastoma when administered in high-dose infusions. Despite inactivity in five transplantable tumor models, thioproline was taken to a clinical trial in Spain; 5 mg/kg thioproline administered po or iv produced regression of some metastatic nodules in a few patients, but only 1/17 patients (a case of renal-cell carcinoma) sustained a true partial remission. Daily ip benzaldehyde at 100 mg/kg produced 40% growth retardation of Adenocarcinoma 775 in mice, and only 10 mg/kg produced a greater than 50% retardation of Ehrlich ascites tumor. Other transplanted tumors were less responsive. No toxicity was observed or expected at these doses. Recent research indicates that retinoids exert direct cytostatic effects on many malignant cell lines in vitro, at concentrations which are not cytotoxic. Sensitivity to this effect appears to be dependent on the presence of retinoic acid-binding protein, and the in vitro efficacy of various retinoids reflects their affinity for this protein. (128 Refs)

3. MECHANISMS OF HUMAN CELL-MEDIATED CYTOTOXICITY. II. CORRECTION OF THE SELECTIVE DEFECT IN NATURAL KILLING IN THE CHEDIAK-HIGASHI SYNDROME WITH INDUCERS OF INTRACELLULAR CYCLIC GMP.

Katz P, Roder JC, Zaytoun AM, Herberman RB, Fauci AS
(c/o Fauci), Natl. Inst. Allergy and Infectious Diseases, NIH, Building 10, Room 11B-13, Bethesda, MD, 20205
J Immunol; 129(1):297-302 1982

In this study, we investigated the modulation by cyclic nucleotides of depressed natural killer (NK) cell activity in two patients with the Chediak-Higashi syndrome (CHS). Patients with CHS have defective NK function that is not attributable to a lack of effector cells or a defect in target cell binding. Because of the previously reported abnormalities of the levels of intracellular cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) in these patients, we investigated the effects of exogenous cGMP or its intracellular inducers on CHS NK activity. This study further underscores the importance of cyclic nucleotides as regulators of normal and abnormal NK activity and may be useful in developing means of increasing defective lytic activity in CHS patients and those individuals with demonstrable abnormalities of natural killing. (24 Refs)

4. POLYAMINES IN MAMMALIAN TUMORS. PART II.

Scalabrino G, Ferioli ME
C.N.R. Centre Res. Cell Pathology, Univ. Milan, 20133 Milan, Italy
Adv Cancer Res; 36:1-102 1982

The role of polyamines in oncology is reviewed. Polyamine concentrations in different lines of cultured neoplastic cells in response to microenvironmental conditions, in relation to growth rate and cell cycle phase, and in relation to cyclic nucleotides, and infection with nononcogenic viruses are described. The patterns of polyamines in human oncology are reviewed with respect to levels in blood, urine, and other physiological fluids; levels of polyamine biosynthetic decarboxylases in human neoplastic tissues; and metabolic conjugation of polyamines and antipolyamine antibodies in neoplastic and normal tissues. Diamine oxidase activity in human and experimental neoplasms and physiological and pharmacological inhibitors of polyamines are also discussed. Polyamine biosynthesis research is likely to lead to promising results because the principal characteristic of the phenotype of neoplasms is the lack, or at least an alteration, of regulation of metabolism, including that of polyamines. At present the role of polyamines in eliciting neoplastic behavior and permitting neoplastic growth is almost totally obscure. Studies of the relationship between polyamines and fibronectin may be important in explaining neoplastic behavior. (493 Refs)

5. LYMPHOKINES: A FORUM FOR IMMUNOREGULATORY CELL PRODUCTS. VOLUME 3. LYMPHOKINES IN MACROPHAGE ACTIVATION.

Pick E, Landy M
Dept. Human Microbiology, Sackler Sch. Medicine, Tel-Aviv Univ., Ramat-Aviv, Tel-Aviv, Israel
Lymphokines 3: 450 pp., 1981.

The role of lymphokines (LK) in macrophage activation (MA) is discussed under the following headings: an introduction to MA, biochemical properties of activated macrophages, enhancement of phagocytosis-associated oxidative metabolism as a manifestation of MA, macrophage plasma membrane enzymes as differentiation markers of MA, a developmental concept for the heterogeneity of macrophages in response to LK and other signals, role of colony-stimulating factors as primary regulators of macrophage functions, lipopolysaccharide-unresponsive mice as a model for analyzing LK-induced macrophage differentiation in vitro, MA and resistance to intracellular infection, the role of cyclic nucleotides in the suppression of intracellular replication of Toxoplasma in LK-activated macrophages, cytotoxicity of activated macrophages for the multicellular parasite Schistosoma mansoni, the role of LK in granulomatous inflammations, the cytostatic and cytocidal effects of macrophages and their specificity for tumor cells, the killing of syngeneic tumor cells by guinea pig macrophages activated in vitro with lymphocyte mediators, tumor cytotoxicity by LK-activated macrophages: development of macrophage tumoricidal activity requires a sequence of reactions, induction of tumoricidal capacities in mouse and rat macrophages by LK, MA to cytotoxicity by the macrophage cytotoxicity (activating) factor, the effect of activated macrophages on tumor growth in vitro and in vivo, and requirement of transmethylation reactions for immune effector function. (1532 Refs)

6. EFFECT OF TOCOPHEROL (VITAMINE E) ACID SUCCINATE ON GROWTH AND MORPHOLOGY OF MOUSE MELANOMA AND MOUSE NEUROBLASTOMA CELLS IN CULTURE (MEETING ABSTRACT).

Prasad KN
University of Colorado Health Sciences Center, Denver, CO, 80262
Proc Am Assoc Cancer Res; 23:900 1982

Vitamin E acid succinate (5 ug/ml) induced morphological alterations (increases in size, long cytoplasmic processes, parallel arrangement of cells) and growth inhibition in mouse melanoma (B-16) cells in culture, but it causes only growth inhibition with enlargement of cells in mouse neuroblastoma (NBP2) cells in culture. Vitamin E acid succinate at a similar concentration was ineffective in mouse fibroblasts (L-cells) in culture. The effects of vitamin E acid succinate were mostly irreversible; however, partially affected cells and vitamin E-resistant cells exist in culture. These cells eventually reach confluency. The addition of sodium succinate with an equivalent volume of ethanol or other forms of vitamin E such as aquasol DL-alpha-tocopherol acetate, DL-alpha-tocopherol nicotinate and DL-alpha-tocopherol free alcohol at a similar concentration was ineffective. The solvents for aquasol vitamin E acetate and vitamin E free alcohol at higher concentrations were toxic; therefore, these forms of vitamin E were considered not suitable for an in vitro study. The effects of vitamin E acid succinate were also expressed in hormone supplemented serum free medium (SFM); however, the concentrations needed to produce changes in SFM were 5 to 10 times less than that needed in serum supplemented medium. Vitamin E also modifies the effect of several tumor therapeutic agents such as cyclic AMP stimulating agents, vincristine, 5-fluorouracil, adriamycin, bleomycin, cis-platinum, and x-irradiation. The extent of modification depends upon the cell type, and particular agent. (no Refs)

7. ACTIVITY OF PHASE I DRUGS HOMOHARRINGTONINE (HHT) AND TRICYCLIC NUCLEOTIDE (TCN) AGAINST SURGICAL EXPLANTS OF HUMAN TUMORS IN THE 6-DAY SUBRENAL CAPSULE (SRC) ASSAY (MEETING ABSTRACT).

Cobb WR, Bogden AE, Reich SD, Griffin TW, Esber HJ
EG and G Mason Research Institute, Worcester, MA, 01608
Proc Am Assoc Cancer Res; 23:877 1982

The feasibility of determining the potential clinical activity of new chemotherapeutic agents while still undergoing Phase I toxicology was examined by testing HHT (NSC-141633) and TCN (NSC-280594) against fresh surgical explants of human tumors in the 6-day in vivo SRC Assay. Twenty tumors of different histologic types (breast, colon, lung, ovary, kidney and lymphoma) were screened against HHT and TCN simultaneously with five chemotherapeutic agents used clinically for treatment of these specific tumor types. Treatment schedule was QD1-5, and the criterion for drug activity was regression in graft size greater than 20%. HHT was active against 7/20 tumors with a response rate of 35%, and TCN active in 11/20 tumors gave a response rate of 55%. Cytoxan (CTX), a standard agent tested concurrently against 16 of the tumors, yielded a 50% response rate. All drugs showed differences in activity within tumors as well as between tumors. Seven of 20 (35%) tumors were unresponsive to all drugs tested, one was responsive only to HHT and TCN, and one was responsive only to TCN. Dose responses to both drugs were obtained with sensitive tumors. Since 1mm3 tumor fragments under the renal capsule more nearly represent micrometastatic disease in terms of tumor burden, the relatively high response rate of TCN compares favorably with CTX as a potential chemotherapeutic agent. (no Refs)

8. STUDIES ON THE MECHANISM OF CELL DEATH IN L1210 CELLS TREATED WITH THE TRICYCLIC NUCLEOSIDE, TCN (MEETING ABSTRACT).

Passiatore JE, Wotring LL, Townsend LB
College of Pharmacy, University of Michigan, Ann Arbor, MI, 48109
Proc Am Assoc Cancer Res; 23:841 1982

Investigation of the mechanism of TCN-induced cell death is especially timely since TCN-5'-phosphate (a prodrug form of the adenosine analog TCN) is currently in Phase I clinical trials for cancer chemotherapy. In L1210 cell cultures treated with 10-6M TCN, the cell number determined using a Coulter counter decreased to 40% of the initial number after 48 hr, while the hemacytometer count was nearly constant. The volume distribution of the TCN-treated cells contained a subpopulation with size below the control range, and below the usual detection threshold of the Coulter counter. After 24 hr the subpopulation contained 22% of the total cell number, and after 48 hr increased to 66%. Flow cytometric analysis of the DNA content of the TCN-treated cells after 24 hr showed 15% of the cells in a discrete subpopulation with DNA content below the control range; 73% after 48 hr. Further study suggested that cell viability decreased before the cells shrank and lost DNA content. Viability as indicated by Nigrosin dye exclusion after 24 hr of TCN treatment was 49% and after 48 hr was only 14%. Similar results were obtained using Eosin Y. The ability of the TCN-treated cells to form colonies was decreased to 17% of control at 24 hr, and 4% at 48 hr. Thus cell viability as measured by either method decreased progressively with time of TCN treatment. In contrast, treatment of L1210 cells with another adenosine analog APPCR [4-amino-3 carboxamido-1-(beta-D-ribofuranosyl)pyrazolo[3,4-d] pyrimidine] (5x10-4M) for 36 hr caused total loss of cell viability as indicated by colony formation while the cells retained the ability to exclude Eosin Y. These results suggest that TCN-induced cell death has a mechanism different from that of APPCR. (1 Ref)

9. INTRA-AORTIC PROSTAGLANDIN E1 (PGE1) INFUSION, PAPAVERINE AND MULTIAGENT CHEMOTHERAPY IN DISSEMINATED NEUROBLASTOMA (MEETING ABSTRACT).

Imashuku S, Sugano T, Fujiwara K, Todo S, Ogita S, Goto Y
Kyoto Prefectural University of Medicine, Kamikyo-Ku 602, Kyoto, Japan
Proc Am Assoc Cancer Res; 23:478 1982

Patients with disseminated neuroblastoma have not sucessfully been cured with intensive chemotherapy and/or radiotherapy. Adenosine 3',5'-monophosphate (cAMP) induces differentiation of neuroblasts in vitro. Clinical application of cAMP-increasing agents, as adjuvant therapy, is worth attempting to promote maturation, thus decreasing tumorigenicity of neuroblastoma, which does not respond to conventional therapy. We found that prostaglandin E1 (PGE1) given simultaneously with papaverine was superior to papaverine alone in increasing cAMP. However, the use of intravenous PGE1 (50-100 ng/kg/min) is limited because of side effects. A 7-year-8-month old boy with Evans' stage III abdominal neuroblastoma, unresectable and biopsy-proven, received intra-aortic infusion of PGE1. A chronofuzer tube was placed for 3 months, through A (Arteria) gastroduodenalis via A hepatica communis, and for another 3 months, through A femoralis profunda via A iliaca externa into the aorta at Th8 level, proximal to the feeding arteries to the tumor. PGE1 was infused with a portable infusion pump without any noticeable side effect. During the period, papaverine (120 mg/day, PO) and multiagent chemotherapy consisting of adriamycin, cyclophosphamide, cis-platinum and

VM26 were given. At second-look surgery, tumors appearing ganglioneuromatous were subtotally resected. Histology disclosed extensively degenerated tumor with marked fibrosis and remaining tumor cells mostly resembled atypical ganglion cells with some small round cells seen focally. (no Refs)

10. INDUCTION OF NORMAL AND METAPLASTIC SQUAMOUS DIFFERENTIATION IN DIVERSE ORGANS BY CYCLIC ADENINE NUCLEOTIDE AND PROSTAGLANDINS: HUMAN BREAST (MEETING ABSTRACT).

Schaefer FV, Custer RP, Sorof S
Institute for Cancer Research, Philadelphia, PA, 19111
Proc Am Assoc Cancer Res; 23:139 1982

Squamous metaplasia is a common abnormality of epithelial cell development and differentiation in many organs of animals and humans. We have found that the combination of dibutyryl cyclic AMP, prostaglandins E1, E2, B1, and papaverine (phosphodiesterase inhibitor) in various culture systems not only accelerates normal epidermization in chick embryo skin but also induces squamous metaplasia and keratinization in mouse mammary glands, mouse prostate glands, and breast tissue of women. Dibutyryl cyclic AMP alone at ten-fold higher concentration is highly effective in mouse prostate glands and human breast tissue. The degree of metaplasia and keratinization induced in the cultured breast tissue of premenopausal women depends on the stage of the menstrual cycle at the time of biopsy. Breast tissue of women in the first 10 days of the cycle responds minimally, while that obtained during the final 10 days, when blood levels of progesterone and estrogen are known to peak, produce extensive squamous metaplasia and keratin plaques. Breast tissue of post-menopausal women yield intermediate levels of induction. Retinylidene dimedone or phorbol didecanoate inhibit the induction process. Our findings with the four organ culture systems suggest that cyclic adenine nucleotide, aided by prostaglandins, may act generally as physiological inducers of normal and metaplastic squamous cell development and differentiation in the epithelia of diverse organs. (1 Ref)

11. CYCLIC AMP INDUCES TS-ALTERATIONS IN MORPHOLOGY IN RAT LIVER EPITHELIAL CELL TS-TRANSFORMANTS (MEETING ABSTRACT).

Niles R, Loewy B, Krah D
Department of Biochemistry, Boston University School of Medicine, Boston, MA, 02118
Proc Am Assoc Cancer Res; 23:102 1982

Cholera toxin via its ability to increase intracellular cyclic AMP levels can induce drastic changes in cell morphology. We have found that a rat liver epithelial cell (TS-223) which is ts for expression of certain transformed properties is also ts for cyclic AMP-induced morphological alterations. TS-223 cells were treated with various concentrations of cholera toxin (CT) or 8-bromocyclic AMP (8BrcA) at either the permissive temperature (37C) or the non-permissive temperature (40.5C). Following a 2-5 hr incubation period, the cells were photographed and processed for cyclic AMP measurements. Both CT and 8BrcA only altered cell shape at the permissive temperature. These changes consisted of extensive cytoplasmic process formation and occasional rounding of the cell body. Shift-up/shift-down experiments indicated that the change in the response occurred rapidly ie within 2 hrs. Cyclic AMP measurements indicated that CT stimulated cyclic AMP production to the same extent at either temperature. Cycloheximide at concentrations which inhibited protein synthesis by 80% did not prevent the acquisition of responsiveness during shift-down experiments. The data suggest the presence of either ts-sensitive cyclic AMP-dependent protein kinases or phosphate-acceptor protein substrates. (no Refs)

REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES

12. INDUCTION OF GLUTAMINE SYNTHETASE BY DIBUTYRYL CYCLIC AMP IN C-6 GLIOMA CELLS.

Browning ET, Nicklas WJ
Dept. Pharmacology, Rutgers Medical Sch., P.O. Box 101, Piscataway, NJ, 08854
J Neurochem; 39(2):336-341 1982

Glutamine synthetase was found to be increased in C-6 glioma cells as a result of increasing culture passage and N-6,2'-O-dibutyryl cyclic AMP (dbcAMP) treatment. At low passage dbcAMP produced a 2.5-fold increase in glutamine synthetase activity per unit of cellular protein. At high passage control glutamine synthetase was approx double that seen at low passage, but dbcAMP produced an additional 65% increase. Lactate dehydrogenase activity was also increased by dbcAMP treatment at both low and high passage, but culture passage produced no change in the lactate dehydrogenase. With increasing culture passage, the ratio of cellular protein to DNA doubled. therefore, expression of data per unit of protein tended to minimize the apparent changes in activity. The max increase in glutamine synthetase activity produced by both dbcAMP and increasing culture passage and expressed on a DNA basis was 5.6-fold. The increase in glutamine synthetase activity was generally linear during the first 20 hr of drug treatment, after which enzyme activity remained nearly constant up to 72 hr. Ninety percent or more of the dbcAMP remained in the medium at the end of 48-hr exposure of cells to dbcAMP. 8-Br-Cyclic AMP also increased glutamine synthetase activity of C-6 cells, but n-butyrate did not. Isoproterenol, which increases cyclic AMP in C-6 cells, increased glutamine synthetase activity. The effect of isoproterenol on glutamine synthetase was inhibited by the beta-adrenergic blocking agent sotalol. Cycloheximide (10 ug/ml) inhibited the dbcAMP effect on glutamine synthetase activity and also decreased the control enzyme activity by 60%. (Author abstract) (32 Refs)

13. EFFECTS OF CYCLIC NUCLEOTIDES ON THE SHEDDING OF TUMOR CELL SURFACE MEMBRANES.

Liepins A, De Harven E
Memorial Univ. Newfoundland, Faculty of Medicine, St. John's, Newfoundland, Canada
Exp Cell Res; 139(2):265-273 1982

Mastocytoma P815 cells can be induced to shed membrane vesicles (MV) from their surfaces at low temperature, a condition known to disrupt microtubules. In the present study we have investigated the effects of various nucleotides on this tumor cell surface behavior. Our data show that the cyclic nucleotides cAMP and cGMP significantly inhibit the low temperature-induced shedding of MV from the tumor cell surfaces. GTP and the phosphodiesterase inhibitor theophylline exhibited similar inhibitory effects. These data are interpreted in terms of nucleotide-induced stabilization of cytoskeletal components. Transmission (TEM) and scanning electron microscopy (SEM) of cells shedding MV revealed that these structures originated from blebs on the cell surface. (Author abstract) (32 Refs)

CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES

14. TROPHOBLASTIC CELLS OF THE HYDATIDIFORM MOLE CONTAIN A B1-SUBTYPE ADRENERGIC RECEPTOR.

Moore JJ, Workman L, Whitsett JA
Neonatology Div., Dept. Pediatrics, Metropolitan General Hosp., 3395 Scranton Road, Cleveland, OH, 44109
J Clin Endocrinol Metab; 55(2):341-346 1982

Both beta-1 and beta2-adrenergic receptors have been previously described in normal human placental homogenates; the cells upon whose surface membranes these receptors reside have not been identified. In order to show that a beta1-adrenergic receptor is present on trophoblastic cells, the cells which mediate maternal-fetal transport and produce placental hormones, beta-adrenergic receptors were demonstrated in membrane fractions of human hydatidiform mole. Microscopic sections of the mole samples used demonstrated edematous villi lined by trophoblastic cells with minimal nontrophoblastic (stromal or vascular) contamination compared with placenta. (-)-[3H]Dihydroalprenolol [(-)-[3H]DHA]binding to molar membranes was reversible and saturable to a single class of sites (Kd = 0.97 +-0.12 nM; n = 7; maximum binding capacity, 72.9 +-6.4 fmol/mg protein). (-)-[3H]DHA binding was associated with catecholamine-stimulated adenylate cyclase activity. Agonist competition for the molar beta-adrenergic receptor showed the order of potency to be (-)isoproterenol much greater than norepinephrine = epinephrine, characteristic of a beta1-adrenergic receptor subtype. Competition for (-)-[3H] DHA binding to trophoblastic membranes by the beta-adrenergic receptor subtype-specific agents metoprolol (beta1 selective) and zinterol (beta2 selective) was also characteristic of a homogenous subtype of beta1-adrenergic receptors. Because beta1-adrenergic receptors alone were seen on trophoblast cells, the beta2-adrenergic receptor in placenta must reside on nontrophoblastic elements (stromal or vascular endothelium). No differences in beta-adrenergic receptor binding were seen related with ploidy (2 or 3 N), the presence or absence of a fetus, or the progression of the mole to choriocarcinoma. Two choriocarcinoma cell lines, BeWo and JEG-3, however, showed no specific (-)-[3H]DHA binding. Human trophoblast contains beta1-adrenergic receptors coupled to catecholamine-sensitive adenylate cyclase, supporting a role for catecholamines in the regulation of placental metabolism. (Author abstract) (26 Refs)

15. ESTABLISHMENT OF GONADOTROPIN-RESPONSIVE MURINE LEYDIG TUMOR CELL LINE.

Rebois RV
Membrane Biochemistry Section, Developmental Metabolic Neurology Branch, National Inst. Neurological and Communicative Disorders and Stroke, NIH, Bethesda, MD, 20205
J Cell Biol; 94(1):70-76 1982

Several clonal Leydig tumor cell lines have been established by adapting the transplantable Leydig tumor, M548OP, to culture. One of these cell lines, MLTC-1, has been characterized with regard to the gonadotropin-responsive adenylate cyclase system. The binding of 125I-labeled human chorionic gonadotropin (hCG) was blocked by excess unlabeled hCG and lutropin (LH) but not by follitropin, thyrotropin, or insulin, indicating the presence of specific receptors for hCG and LH. Based on the specific binding of hCG to isolated MLTC-1 membranes, the calculated dissociation constant was 1.0 +-0.2 x 10(-10) M. The receptors appeared identical to those from normal murine Leydig cells when analyzed by SDS PAGE and sucrose density gradient centrifugation. The molecular wt and sedimentation coefficient were 95,000 daltons and 8.5 S, respectively. MLTC-1 cells responded to hCG by accumulating cyclic AMP and producing progesterone. Cyclic AMP accumulation was time- and dose-dependent with a maximal accumulation occurring at approx 0.2 nM hCG. At saturating levels of hCG, cAMP levels reached a max by 30 min and then declined very slowly. Adenylate cyclase activity in membranes prepared from MLTC-1 cells was stimulated by hCG, LH, NaF, cholera toxin, and guanyl-5'-yiimidodiphosphate. Additionally, choleragen was found to ADP-ribosylate a membrane protein of 54,000 daltons. This protein resembles the proposed guanine nucleotide regulatory component in both size and choleragen-dependent reactivity. These data suggest that MLTC-1 cells possess a gonadotropin-responsive adenylate cyclase system consisting of a specific hormone receptor, a regulatory component, and a catalytic subunit. (Author abstract) (40 Refs)

16. PROSTAGLANDIN A1 INHIBITS HUMAN MELANOMA COLONY FORMING UNITS (MCFU) IN SOFT AGAR (MEETING ABSTRACT).

Bregman MD, Sander D, Kramb P, Meyskens FL, Neifeld JP
Department of Internal Medicine, University of Arizona Health Sciences Center, Tucson, AZ, 85724
Proc Am Assoc Cancer Res; 23:935 1982

Prostaglandins (PG) are potent modulators of cellular growth. Their effect on human tumor stem cells has not been previously reported. We tested the effect of PG on MCFU from biopsies of melanoma tissue. PGA1 (5ug/ml) reduced MCFU by greater than 70% in 5 patients. The dose response curve for inhibition by PGA1 is steep between 0.5 and 5ug/ml. PGF2 alpha was ineffective at these concentrations. Similar results were obtained with cells from human and murine melanoma cell lines, suggesting that detailed experiments with human cell lines may have applicability to the in vivo state. Using a human melanoma cell line M82SS (developed in this laboratory), we found that exposure time was an important parameter. Pre-treatment of these cells for 20 minutes with PGA1 did not result in the inhibition of colony formation which continuous exposure produced. Cyclic nucleotide levels in M82SS were measured to determine if cAMP and cGMP (cyclic guanosine monophosphate) were involved in the inhibition of colony formation. PGA1 may be modulated via a non-cyclic nucleotide mechanism. We have also measured the ability of other hormones and drugs to lower either the dose level and/or the exposure time needed for PG inhibition of MCFU. (no Refs)

17. CLOTTING FACTORS (THROMBIN, FACTOR XIII, FIBRONECTIN) AS REGULATORS OF FIBROBLAST, ENDOTHELIAL CELL AND TUMOR CELL PROLIFERATION (MEETING ABSTRACT).

Bernsmeier R, Pohl J, Bruhn HD
I Medizinische Universitats-Klinik Kiel, Kiel, W. Germany
Onkologie; 4(5):248 1981

Thrombin and factor XIII stimulate fibroblast proliferation during a simultaneous intracellular increase in cyclic guanosine monophosphate; fibronectin on the other hand inhibits the relative 3H-thymidine uptake of fibroblasts. More recent research has been able to demonstrate that vascular wall endothelial cells are stimulated in a similar fashion by thrombin and factor XIII. Comparison studies with tumor cells (HeLa, sarcoma cells) indicate that at least in some cases tumor cells are also subject to the regulations mentioned. Incubating fibroblasts with neuraminidase completely eliminates thrombin activity and factor XIII action is shifted to higher concentrations suggesting that neuraminidase either splits a fragment off a receptor or alters the receptor's conformation. (no Refs) (In GER)

18. HISTAMINE-INDUCED CYCLIC AMP RESPONSE IN ACUTE LEUKAEMIA.

Maurer WM, Latz D
Ludwig Boltzmann-Inst. for Leukemia Res. and Hematology, Vienna, Austria
IRCS Med Sci [Cancer]; 9(10):1000 1981

The histamine induced synthesis of cyclic AMP in the adenylate cyclase system was studied to evaluate a possible role of histamine in acute leukemia. Fourteen patients with acute leukemia were evaluated for histamine binding prior to treatment. Basal cyclic AMP (cAMP) content measured without histamine ranged from 7.8-77 picomoles (pmol) in acute lymphocytic leukemia (ALL) and 3.8-29.7 pmol in acute myelocytic leukemia (AML). Histamine induced stimulation of cAMP synthesis was 5.4-fold in common ALL; poor or no stimulation was found in AML and in one patient with ALL which was diagnosed as T-cell ALL. (6 Refs)

19. EFFECT OF GLUCAGON ON AMINO ACID TRANSPORT AND CYCLIC ADENOSINE 3':5'-MONOPHOSPHATE PRODUCTION IN RAT HEPATOMA CELL LINE MCA-RH 8994 IN CULTURE.

Kelley DS
Dept. Biochemistry, West Virginia Univ., Sch. Medicine, Morgantown, WV, 26506
Cancer Res; 42(8):3116-3119 1982

The rates of alpha-aminoisobutyrate (AIB) transport and cyclic adenosine 3':5'-monophosphate (cAMP) production in response to glucagon (2 x 10(-7) M) have been determined in monolayer cultures of hepatoma cell line 8994 (refes to hepatoma cell line McA-RH 8994) under three different calcium treatments. The calcium treatments were: (a) control, cultures maintained in calcium-containing medium without prior calcium depletion; (b) calcium depleted, cultures maintained in calcium-free medium after treatment with ionophore A23187 and ethylene glycol bis(beta-aminoethyl ether)-N,N'-tetraacetic acid; (c) calcium repleted, cultures maintained in calcium-containing medium after treatment with A23187 and ethylene glycol bis (beta-aminoethyl ether)-N,N'-tetraacetic acid. In the control cultures, glucagon in the absence of methylisobutylxanthine (MIX) has no effect on the rate of AIB transport. AIB transport is approx doubled when glucagon is added in the presence of MIX. Calcium depletion and calcium repletion enhance the rate of AIB transport by about 50 and 100%, respectively. Glucagon and N6,O2-dibutyryl cyclic adenosine 3';5'-monophosphate (1 x 10(-4) M) further increase the rate of AIB transport by 10% in calcium-depleted cultures and by 50% in calcium-repleted cultures. The stimulation of AIB transport by glucagon as well as by the ionophore can be blocked by the simultaneous addition of cycloheximide (0.1 mM) or actinomycin D (3.2 uM). In the absence of MIX, there is about a two-fold increase in the level of cAMP in response to glucagon. In the presence of MIX, glucagon causes more than 100-fold increase in the level of cAMP. The presence or absence of calcium does not affect the basal or glucagon-stimulated production of cAMP in these cultures. We have reported previously that, in monolayer cultures of rat hepatocytes, calcium repletion enhanced the glucagon stimulation of AIB transport while it markedly inhibited the production of cAMP in response to glucagon. It is suggested that calcium rather than cAMP has a direct effect on the amino acid transport system. (Author abstract) (24 Refs)

20. ALTERATIONS IN THE SUBCELLULAR DISTRIBUTION OF GUANYLATE CYCLASE AND ITS RESPONSIVENESS TO NITRIC OXIDE IN DIETHYLSTILBESTROL-INDUCED RENAL TUMORS.

Braughler JM, Gilloteaux J, Steggles AW
Dept. Pharmacology, Northeastern Ohio Univ. Coll. Medicine, Rootstown, OH, 44272
Cancer; 50(1):78-84 1982

The cyclic GMP content of diethylstilbestrol-induced renal tumors in the male golden hamster was increased nearly 130-fold over that in kidney from control animals. Cyclic GMP in tumors was 91.80 +-19.18 pmoles cyclic GMP/mg protein compared to 0.72 +-0.07 in control kidneys. Cyclic AMP in tumors was also increased over control, however, to a much lesser degree (2.7-fold). In control kidneys, 84.6% of homogenate guanylate cyclase activity was recovered in the 100,000 x g supernatant fraction. Total homogenate guanylate cyclase activity from diethylstilbestrol-induced renal tumors was increased 5.5-fold over that in control kidneys and only 8.1% was associated with the 100,000 x g supernatant fraction. Neither the soluble or particulate guanylate cyclase from renal tumors could be activated by nitric oxide. The unresponsiveness of tumor guanylate cyclase to nitric oxide was independent of the cation cofactor, and not due to a shift in the dose response curve for nitric oxide. Responsiveness to nitric oxide was not restored by thiols, sugars, other proteins, or hemoglobin. Basal cyclic AMP formation by soluble guanylate cyclase from renal tumors was dramatically increased over that observed in control kidneys, and could not be increased further by nitric oxide. This is the first study of cyclic GMP and guanylate cyclase in a primary estrogen-induced tumor. The possibility that the changes observed in guanylate cyclase from diethylstilbestrol-induced renal tumors are related to in vivo activation of the enzyme by epoxide metabolites of diethylstilbestrol is discussed. (Author abstract) (31 Refs)

CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS

21. EFFECTS OF PHORBOL MYRISTATE ACETATE AND A LYMPHOKINE ON CYCLIC 3':5'-GUANOSINE MONOPHOSPHATE LEVELS AND PROLIFERATION OF MACROPHAGES.

Hadden EM, Sadlik JR, Coffey RG, Hadden JW
Dept. Pharmacology, Univ. South Florida Medical Center, Tampa, FL, 66312
Cancer Res; 42(8):3064-3069 1982

The tumor promoter phorbol myristate acetate (PMA) was compared to a lymphokine macrophage mitogenic factor (MMF) for its ability to induce replication of guinea pig peritoneal and alveolar macrophages. Like MMF, PMA induces

DNA synthesis of both cell populations with peak thymidine incorporation at 72 hr of culture. Optimal concentrations of PMA for the peritoneal and alveolar cells were 1.6 x 10(-7) and 1.6 x 10(-9) M, respectively. the magnitude of the effect is slightly less than MMF but greater than that of phytohemagglutinin or concanavalin A. Indomethacin added to inhibit prostaglandin synthesis potentiates the effects of MMF but has little effect on the actions of PMA and the other mitogens. Potentiation by indomethacin of the effects of PMA on the peritoneal cell was observed only at the suboptimal concentration of PMA (1.6 x 10(-8) M). By adherence criteria and density gradient fractionation, the cell responding to PMA is confirmed to be the macrophage. Cell counts and nuclear radioautography confirm that replication in this system is reasonably well reflected by thymidine incorporation. The effects of PMA and its analogs as macrophage mitogens correlate with their tumor-promoting effects. Both PMA and MMF induce early increases in peritoneal macrophage levels of cyclic 3':5'-guanosine monophosphate without changes in the levels of cyclic 3':5'-adenosine monophosphate. These studies indicate that PMA offers a useful probe of macrophage function. (Author abstract) (41 Refs)

22. PREVENTION OF PLATELET AGGREGATION IN PATIENTS WITH PROSTATIC CANCER DURING ESTROGEN THERAPY.

Koiso K, Akima H, Niijima T
7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan
Urology; 19(6):579-583 1982

In prostatic cancer patients treated with synthetic estrogen, hexestrol, increased levels of platelet aggregation were observed in accordance with a significant decrease of platelet cyclic AMP. Dipyridamole, which is known to inhibit phosphodiesterase activity, was used to prevent this platelet aggregating effect of hexestrol. Inhibitory effect of dipyridamole on platelet aggregation was demonstrated in patients treated with hexestrol during 1 yr of observation. (Author abstract) (18 Refs)

PROTEIN KINASE STUDIES

23. DIRECT ACTIVATION OF CALCIUM-ACTIVATED, PHOSPHOLIPID-DEPENDENT PROTEIN KINASE BY TUMOR-PROMOTING PHORBOL ESTERS.

Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa U, Nishizuka Y
(c/o Nishizuka) Dept. Biochemistry, Kobe Univ. Sch. Medicine, Kobe 650, Japan
J Biol Chem; 257(13):7847-7851 1982

Tumor-promoting phorbol esters such as 12-O-tetradecanoylphorbol-13-acetate (TPA) directly activate in vitro Ca++-activated, phospholipid-dependent protein kinase (protein kinase C), which normally requires unsaturated diacylglycerol. Kinetic analysis indicates that TPA can substitute for diacylglycerol and greatly increases the affinity of the enzyme for Ca++ as well as for phospholipid. Under physiological conditions, the activation of this enzyme appears to be linked to the receptor-mediated phosphatidylinositol breakdown which may be provoked by a wide variety of extracellular messengers, eventually leading to the activation of specific cellular functions or proliferation. Using human platelets as a model system, TPA is shown to enhance the protein kinase C-specific phosphorylation associated with the release reaction in the total absence of phosphatidylinositol breakdown. Various phorbol derivatives which have been shown to be active in tumor promotion are also capable of activating this protein kinase in vitro systems. (Author abstract) (39 Refs)

24. PURIFICATION OF A TYROSINE-SPECIFIC PROTEIN KINASE FROM ROUS SARCOMA VIRUS-INDUCED RAT TUMOR.

Blithe DL, Richert ND, Pastan IH
Lab. Molecular Biology, NIH, NCI, Bethesda, MD, 20205
J Biol Chem; 257(12):7135-7142 1982

We have identified a tyrosine kinase activity present in tumors which were raised in rats by subcutaneous injection of Rous sarcoma virus-transformed rat cells (SR-NRK). This kinase phosphorylates tyrosine on the heavy chain of IgG from tumor-bearing rabbit (TBR) sera specific for the src gene product, pp60src. Using TBR-IgG phosphorylation as an assay, we have purified this kinase over 7,200-fold. The purification procedure involves detergent extraction of tumors followed by sequential column chromatography on hydroxylapatite, DEAE-Sephacel, oligodeoxyadenosine-cellulose, an affinity column prepared from TBR-sera, and Sephacryl S-200. The IgG kinase activity behaves as a molecule of apparent Mr = 54,000 on Sephacryl S-200 molecular sieve chromatography. Analysis of the Sephacryl fractions by SDS-PAGE indicates that a major Coomassie blue-stained band with an apparent Mr = 54,000 (p54), co-elutes with the peak of kinase activity. From 600 g of tumors, approx 200 ug of p54 are obtained. We have four types of evidence which show that p54 is related to pp60src. (1) Purified p54 is capable of undergoing endogenous phosphorylation in the presence of [gamma-32P]ATP producing a 32P-labeled pp54 polypeptide which is specifically immunoprecipitated by TBR-sera and contains only phosphotyrosine. (2) Purified p54 competes with 32P labeled pp60src for binding to TBR-IgG, indicating a degree of purification over starting material which agrees very well with the results obtained by the IgG kinase assay. (3) V8 protease digestion of pp60src and p54 suggests that they share a common 26,000 fragment. (4) Antibodies to partially purified p54 specifically precipitate pp60src from Rous sarcoma virus-transformed chicken cells. (Author abstract) (45 Refs)

25. INHIBITION OF CELLULAR IMMUNITY IN SMALL CELL BRONCHOGENIC CARCINOMA OF THE LUNG: CHARACTERIZATION OF LOW-MOLECULAR SERUM FACTORS WHICH INHIBIT PROTEIN SYNTHESIS IN NORMAL LYMPHOCYTES (MEETING ABSTRACT).

Manke HG, Drings P, Lenhard V, Dirks HP, Toomes H
Onkologisches Zentrum Heidelberg-Mannheim, Mannheim, W. Germany
Onkologie; 4(5):266 1981

The synthesis of RBC-receptor proteins, a differentiation marker of human peripheral T-lymphocytes, is inhibited in patients with small cell bronchogenic carcinoma. This glycoprotein, which has been briefly characterized by Owen and Fanger as well as by Gurtler persists in the membrane for different periods of time depending on the T-cell population. When protein synthesis, nucleocytoplasmic mRNA transfer and mRNA synthesis are inhibited, normal T-lymphocytes are become depleted of RBC-receptor protein with characteristic temporal kinetics. In small cell bronchogenic carcinoma there is a very profound inhibition of the cellular immune system. In a study of 32 patients with small cell bronchogenic carcinoma the defect in cellular immunity correlated with a decrease in

RBC-rosette forming cells and patient lymphocyte proliferation in response to mitogenic stimulation. Serum and serum fractions from patients with low RBC-rosette formation inhibited the expression of RBC-receptor protein by normal human peripheral lymphocytes within 60 min when incubated at 37C. This inhibition coincided with a reduction in protein synthesis (H3-amino acid uptake). The inhibitory serum fraction was further purified by means of high pressure liquid chromatography; the inhibitory trypsin-sensitive fraction lies in the 20,000 dalton region. The present studies refer to an inhibition of protein synthesis possibly on account of induction of a cyclic AMP-dependent protein kinase. (no Refs) (In GER)

26. INDUCTION OF CYCLIC AMP-DEPENDENT PHOSPHORYLATION IN CYTOSOL OF DIFFERENTIATING MOUSE ERYTHROLEUKEMIA CELL LINE 179.

Emler CA, Fontana JA, Butcher FR
(c/o Butcher) Dept. Biochemistry, West Virginia Univ. Medical Center, Morgantown, WV, 26506
FEBS Lett; 142(2):210-214 1982

The effects of calcium and cyclic AMP (cAMP) on the in vitro phosphorylation of proteins in a soluble fraction from mouse erythroleukemia (MEL) cells at various times during the process of differentiation were studied. A marked decrease in phosphorylation of a 120,000-mol wt protein occurred with the addition of dimethylformamide that was independent of cAMP and calcium. A specific cAMP-dependent protein phosphorylation at mol wt 70,000 occurred within 24 hr of addition of inducer, regardless of which inducer was used. Early cAMP-dependent protein phosphorylation events are crucial in the differentiation process. (18 Refs)

27. ADENOSINE 3,5'-CYCLIC MONOPHOSPHATE DEPENDENT AND INDEPENDENT PROTEIN KINASE ACTIVITIES IN 1,2-DIMETHYLHYDRAZINE INDUCED RAT COLON CANCER.

Singh D, Loven DP, Stevens RH
Dept. Anatomy, Radiation Res. Lab., University Iowa, Iowa City, IA, 52242
Chem Biol Interact; 40(2):169-175 1982

The adenosine 3',5'-cyclic monophosphate (cAMP)-dependent and cAMP-independent kinase activities were measured in the 1,2-dimethylhydrazine (DMH) induced rat colon cancer and in untreated colon. Previous studies had shown that intestinal tumors induced by chronic exposure to DMH contained two-fold less intracellular cAMP. The present findings indicate that reduction in cAMP-dependent protein kinase activities also occur in colon cancer cells. Similar hydrogen ion dependence (pH 6-7) and approximate association constants (Ka approx 0.1 uM) were observed for the enzymes existing in both normal and tumor tissues, while the cAMP-dependent tumor protein kinase was found to phosphorylate phosvitin and casein to a greater degree. These recent findings are consistent with the concept that the concentrations of cAMP and activities of its associated enzyme system are inversely related to the cell proliferation state. (Author abstract) (22 Refs)

28. POLYAMINES IN BIOLOGY AND MEDICINE.

Morris DR, Marton LJ
Dept. Biochemistry, Univ. Washington Sch. Medicine, Seattle, WA
Biochem Dis 8: 459 pp., 1981.

The biosynthesis, metabolism, and biological function of polyamines (PA) and their relationships to clinical medicine are discussed. Chapters related to PA biosynthesis and metabolism include discussions of the following topics: biosynthesis of putrescine, aminopropyl group transfers in PA biosynthesis, inhibition of the purification and characterization of spermine synthase from bovine brain by 5'-methylthioadenosine, biosynthesis and metabolism of PA in plants, control of ornithine decarboxylase activity in prokaryotic and eukaryotic cells, transcriptional induction by trophic hormones via cyclic AMP and cyclic AMP-dependent protein kinase pathway of ornithine decarboxylase (OD), amide-bond-forming reactions of PA, oxidation of PA and diamines, and turnover of PA. Chapters on the biological function of PA include consideration of interactions of PA with polynucleotides, PA and protein synthesis; in vivo studies of the roles of putrescine and spermidine in Escherichia coli, biochemistry, specificity, and biological effects of inhibitors of PA synthesis, consideration of the use of ornithine analogs, PA and the control of the cell cycle in animal cells, and potential use of animal cell mutants in PA research. Also discussed are approaches to the study of PA as cancer markers, clinical aspects of PA in nonneoplastic conditions, diamine oxidase (histaminase) activity in human disease, PA metabolism in humans, the PA-pyridoxal-phosphate paradigm, inhibition of S-adenosylmethionine decarboxylase as an approach in cancer therapeutics, and potential use of OD inhibitors as therapeutic agents. (129 Refs)

29. CYTOSKELETAL TARGETS FOR VIRAL TRANSFORMING PROTEINS WITH TYROSINE PROTEIN KINASE ACTIVITY.

Sefton BM, Hunter T, Nigg EA, Singer SJ, Walter G
Tumor Virology Lab., Salk Inst., San Diego, CA, 92138
Cold Spring Harbor Symp Quant Biol; 46(part2):939-951 1981

The phosphoamino acid compositions of several cytoskeletal proteins from both normal and transformed cells were analyzed in order to see if any of the proteins could be substrates for pp60src, which is localized in the cytoplasm of transformed cells. Vinculin, one of the proteins studied, contains increased levels of phosphotyrosine in Rous sarcoma virus-transformed cells and therefore may be a primary substrate for pp60src. (80 Refs)

30. MOLECULAR EVENTS IN CAMP-MEDIATED REVERSE TRANSFORMATION.

Lockwood AH, Trivette DD, Pendergast M
Dept. Anatomy, Univ. North Carolina Sch. Medicine, Chapel Hill, NC, 27514
Cold Spring Harbor Symp Quant Biol; 46(part2):909-919 1981

Studies of mouse BALB/c 3T3 and Chinese hamster ovary cells demonstrated that phosphorylation and dephosphorylation of specific cytoskeletal and cytoplasmic proteins occur during reverse transformation of transformed cells by cyclic (c)AMP. The phosphoproteins are thought to mediate the acquisition of normal morphology and the organization of the cytoskeleton induced in transformed cells by cAMP. (40 Refs)

OTHER RELATED STUDIES

31. ALTERATIONS OF GLIAL TUMOR CELL CA2+ METABOLISM AND CA2+-DEPENDENT CAMP ACCUMULATION BY PHORBOL MYRISTATE ACETATE.

Brostrom MA, Brostrom CO, Brotman LA, Lee CS, Wolff DJ, Geller HM
Dept. Pharmacology, Rutgers Medical Sch., Univ. Medicine and Dentistry New Jersey, Piscataway, NJ, 08854
J Biol Chem; 257(12):6758-6765 1982

C6 glial tumor cells exposed to phorbol myristate acetate (PMA) possessed lowered cAMP content, reduced ability to accumulate cAMP in response to norepinephrine or cholera toxin, and a three-fold increase in the concentration of norepinephrine producing 50% of the maximal rate of cAMP accumulation. Detectable effects on cAMP accumulation occurred within 10 min of exposure to PMA, and prominent effects by 2 hr. PMA similarly affected cells pretreated with cycloheximide. In contrast, Ca++-depleted preparations of control and PMA-treated cells accumulated cAMP identically in response to norepinephrine or cholera toxin. Ca++ restoration, which increased the rate of cAMP accumulation in control cells severalfold, did not enhance cAMP accumulation in PMA-treated cells. Neither high catecholamine nor high extracellular Ca++ concentrations reversed the suppression of cAMP accumulation by PMA. Trifluoperazine, which inhibited the Ca++-dependent component of norepinephrine-stimulated cAMP accumulation in control cells, did not significantly reduce norepinephrine-stimulated cAMP accumulation in PMA-treated cells. Cell-free preparations of control and PMA-treated cultures did not differ significantly in calmodulin content or in Ca++-stimulated adenylate cyclase, Ca++-dependent cAMP phosphodiesterase, and (Ca++-Mg++)-ATPase activities. The Ca++ content, however, of intact cells decreased with time of PMA treatment. Within minutes after exposure to PMA, the ability of Ca++-depleted cells to take up 45Ca was significantly reduced. Both 45Ca uptake and Ca++-dependent cAMP accumulation were reduced over the same PMA concentration range. (Author abstract) (34 Refs)

32. MECHANISM OF ACTION OF DEOXYADENOSINE/ ADENOSINE DEAMINASE INHIBITOR COMBINATIONS: LYMPHOTOXICITY IN G1 AND G0 PHASES OF CELL CYCLE (MEETING ABSTRACT).

Fox RM, Kefford RF
Ludwig Institute for Cancer Research (Sydney Branch), University of, Sydney Sydney, New South Wales 2006, Australia
Proc Am Assoc Cancer Res; 23:740 1982

ADA inhibitors are undergoing clinical trial in management of T ALL (acute lymphocytic leukemia) and other lymphoid malignancies. Cultured T ALL are sensitive to uM dAdo (deoxyadenosine) in the presence of ADA (adenosine deaminase) inhibitors (EHNA or deoxycoformycin). Using flow cytometry and BdUrd (dibutryl deoxyuridine) quenching of 33343 Hoechst DNA fluorescence we show dAdo (3uM) causes a G1 block in T ALL cells. Cells in S phase exposed to dAdo complete that S phase, pass through G2, + M, enter an accumulate in G1. This phenomenon is prevented by coincubation with dCyd (deoxycytidine) which competes with dAdo for phosphorylation by dAko kinase. The dAdo sensitivity of T ALL cells correlates with dATP accumulation but not with dAdo inhibition of S Adenosyl-homocysteine Hydrolase. Peripheral blood lymphocytes in G0 exposed to uM dAdo elevate their dATP pools to a similar extent as T ALL cells. Progressive cell death was observed after 24 hr. Peripheral blood lymphocytes (T and nonT) and T ALL lack an ecto-adenosine triphosphatase which is present in EBV transformed B lymphocytes. This enzyme can degrade dATP. Mitogen transformed B but not T lymphocytes express this enzyme activity. This specific toxicity of dATP for lymphocytes in G0/G1 is not compatible with inhibition of ribonucleotide reductase. This cycle specific toxicity is analogous to that produced by glucocorticoids or dibutyryl cyclic AMP. The exact biochemical mechanism of dAdo induced cytotoxicity remains to be determined. (no Refs)

33. THE TRANSFORMED CELL.

Cameron IL, Pool TB
Dept. Anatomy, Univ. Texas Health Science Center at San Antonio, San Antonio, TX, 78284
The Transformed Cell. New York, Academic Press, 435 pp., 1981.

Differences between normal and neoplastic transformed cells are described. Topics discussed include a brief description of terminology and basic concepts; background material related to evidence for and against the possible correlation of in vivo tumorigenicity to in vitro changes in the cytoskeletal system, anchorage-dependent growth, plasminogen activator production, agglutinability by lectins, and cell surface and plasma membrane properties; general aspects of regulation of cell proliferation and the relationships between ion movement and energy metabolism in normal and transformed cells; viral transformation of cells; and selected cellular properties, including cyclic nucleotides, polyamine metabolism, mobility of cellular water, cell viscosity, intracellular pH, and element concentration. (1490 Refs)

34. EFFECT OF ACRYLAMIDE ON ACETYLCHOLINESTERASE AND NEURON SPECIFIC ENOLASE ACTIVITY IN MOUSE NEUROBLASTOMA CELLS (MEETING ABSTRACT).

Krause D, Nardone RM
Catholic University of America, Washington, DC, 20064
In Vitro; 18(3,part2):325 1982

A study of the effect of three acrylamides on mouse neuroblastoma cells (NIE-115) and chick brain cell aggregates has revealed a good correlation between the in vivo and the in vitro toxicity ranking of these neurotoxic chemicals. This avenue of investigation has been expanded to include the effect of acrylamide on neuronal endpoints (acetylcholinesterase (AChe) and neurospecific enolase activity) and non-neuronal endpoints (cell survival, proliferation, and enolase activity) in proliferating and dibutyryl c-AMP differentiated NIE-115 cells. Forty-eight hour survival and proliferation of the cells showed a graded dose response. A concentration of 0.01 mM reduced proliferation rate by 10% while 5 mM was completely inhibitory. The AChE activity was unaffected (120 umol product formed/min/mg protein). The AChE activity level was six-fold higher in the differentiated cells and was unaffected by the doses of acrylamide which were used. On the other hand, when survival was used as an endpoint, the differentiated cells showed increased sensitivity; a dose of 3 mM for 48 h was lethal to the culture. Exposure to 0.5 mM for 48 h resulted in a 50% reduction in the activity of neuron-specific enolase while the activity of non-neuron specific enolase was unaffected. (no Refs)

AUTHOR INDEX WITH CITATION NUMBERS

Akima H. 22
Bernsmeier R. 17
Blithe DL 24
Bogden AE 7
Braughler JM 20
Bregman MD 16
Brostrom CO 31
Brostrom MA 31
Brotman LA 31
Browning ET 12
Bruhn HD 17
Butcher FR 26
Cameron IL 33
Castagna M. 23
Cobb WR 7
Coffey RG 21
Custer RP 10
De Harven E. 13
Dirks HP 25
Drings P. 25
Emler CA 26
Esber HJ 7
Fauci AS 3
Ferioli ME 4
Fontana JA 26
Fox RM 32
Fujiwara K. 9
Geller HM 31
Gilloteaux J. 20
Goto Y. 9
Griffin TW 7
Hadden EM 21
Hadden JW 21
Herberman RB 3
Hunter T. 29
Imashuku S. 9
Kaibuchi K. 23
Katz P. 3
Kefford RF 32
Kelley DS 19
Kikkawa U. 23
Koiso K. 22
Krah D. 11
Kramb P. 16
Krause D. 34
Landy M. 5
Lee CS 31
Lenhard V. 25
Liepins A. 13
Lockwood AH 30
Loewy B. 11
Loven DP 27
Lutz D. 18
Manke HG 25
Marton LJ 28
Maurer WM 18
McCarty MF 2
Meek WD 1
Meyskens FL 16
Moore JJ 14
Morris DR 28
Nardone RM 34
Neifeld JP 16
Nicklas WJ 12
Nigg EA 29
Niijima T. 22
Niles R. 11
Nishizuka Y. 23
Ogita S. 9
Passiatore JE 8
Pastan IH 24
Pendergast M. 30
Pick E. 5
Pohl J. 17
Pool TB 33
Prasad KN 6
Rebois RV 15
Reich SD 7
Richert ND 24
Roder JC 3
Sadlik JR 21
Sander D. 16
Sano K. 23
Scalabrino G. 4
Schaefer FV 10
Sefton BM 29
Singer SJ 29
Singh D. 27
Sorof S. 10
Steggles AW 20
Stevens RH 27
Sugano T. 9
Takai Y. 23
Todo S. 9
Toomes H. 25
Townsend LB 8
Trivette DD 30
Walter G. 29
Whitsett JA 14
Wolff DJ 31
Workman L. 14
Wotring LL 8
Zaytoun AM 3

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In this issue:

- **REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES** **1-8**
- **REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES** **9-12**
- **CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES** **13-16**
- **CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS** **17-27**
- **CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES** **28-33**
- **PROTEIN KINASE STUDIES** **34-35**
- **OTHER RELATED STUDIES** **36-39**

REGULATION OF CELL GROWTH AND DIFFERENTIATION BY CYCLIC NUCLEOTIDES

1. DIBUTYRYL CYCLIC AMP TREATMENT MIMICS OVARIECTOMY: NEW GENOMIC REGULATION IN MAMMARY TUMOR REGRESSION.

Huang FL, Cho-Chung YS
Cellular Biochemistry Section, Lab. Pathophysiology, NCI, Bethesda, MD

Biochem Biophys Res Commun; 107(1):411-415 1982

The possibility that the regression process of hormone-dependent tumors is regulated by a transcriptional event and that the regressions produced by hormone removal and dibutyryl cyclic (dc)AMP treatment are evoked by similar or identical mechanisms was investigated. The study presents the first evidence of differential gene expression during growth and regression of hormone-dependent dimethylbenz(a)anthracene-induced rat mammary carcinoma. Changes in translation products demonstrable during regression appeared to be specifically related to the hormone-independence of the tumors. As early as 6 hr post ovariectomy or dcAMP treatment (10 mg/200 g rat/

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day, sc), when there was no appreciable change in tumor size, the in vitro synthesis of several polypeptides instructed by poly(A)RNAs of tumors was either depressed or stimulated, indicating that these changes in gene expression are an early event in tumor regression. Both dcAMP and ovariectomy induced the same changes in the translation products. Thus estrogen and cyclic AMP may counteract each other at the nuclear level in the growth control of hormone-dependent mammary tumors. (17 Refs)

2. GENERAL PROCESS OF INDUCTION OF SQUAMOUS METAPLASIA BY CYCLIC ADENINE NUCLEOTIDE AND PROSTAGLANDINS: MOUSE PROSTATE GLANDS.

Schaefer FV, Custer RP, Sorof S
(c/o Sorof) Inst. Cancer Res., Fox Chase Cancer Center, 7701 Burholme Ave., Philadelphia, PA, 19111
Cancer Res; 42(9):3682-3687 1982

Squamous metaplasia is a common abnormality of cellular development and differentiation in the epithelia of many organs, including the prostate gland in humans. Agents known to elevate the level of intracellular cyclic adenine nucleotide have been found to direct various kinds of epithelial cells onto a pathway of epidermoid (squamous) development and a differentiation marked by keratin production. We report here that the mixture of the cyclic adenine nucleotide; N6,O2'-dibutyryladenosine cyclic 3':5'-monophosphate (0.1 mM); prostaglandins E1, E2, and B1 (each 5 ug/ml); and papaverine (1 uM) induces extensive squamous metaplasia and keratin production in the epithelial cells of mouse ventral prostate glands cultured for 3 wk. The components of the mixture act synergistically. A 10-fold higher concentration of N6,O2'dibutyryladenosine cyclic 3':5'-monophosphate (1 mM) alone is as effective as is the mixture. The presence of either the retinoid, retinylidene dimedone, or the phorbol ester, phorbol-12,13-didecanoate, at 1 uM completely prevents the induction of squamous metaplasia. The results suggest that cyclic adenine nucleotide and indirectly prostaglandins E1 and E2 may play important roles in the spontaneous and exogenously induced production of squamous metaplasia. These same inducing agents have also been found in other studies to bring about squamous metaplasia and keratinization in culture in the mammary glands of mice and the breast tissue of humans, as well as to accelerate normal epidermization in chick embryo skin. The findings in prostate gland, mammary glands, and skin derived from embryonic or adult animals and humans, spanning three species and two classes of vertebrates, suggest that cyclic adenine nucleotide augmented by prostaglandins E1 and E2 may act generally on diverse types of epithelia to bring about normal and metaplastic squamous cell development and a differentiation marked by keratin production. (Author abstract) (50 Refs)

3. INDUCTION OF GP-2 MESSENGER RNA IN F9 CELLS BY RETINOIC ACID AND DIBUTYRYL CYCLIC AMP (MEETING ABSTRACT).

Durkin ME, Chung AE
University of Pittsburgh, Pittsburgh, PA, 15260
Fed Proc; 41(3):683 1982

The nullipotent embryonal carcinoma cell line F9 can be induced to undergo differentiation when treated with retinoic acid and dibutyryl cyclic AMP. After 48 hours of growth in medium containing 2 x 10-7M retinoic acid and 1mM dibutyryl cyclic AMP, the synthesis of the basement membrane-related glycoproteins GP-1, GP-2 (laminin), and entactin is switched on in F9 cells. The increase of GP-2 synthesis has been demonstrated by immunoprecipitation of GP-2 from cell lysates and growth medium of induced cells. Messenger RNA was extracted from induced and uninduced cells and used to program a rabbit reticulocyte lysate in vitro translation system. When translation products directed by induced F9 RNA were immunoprecipitated with GP-2 antiserum and the immunoprecipitates analyzed by SDS (sodium dodecyl sulfate)-polyacrylamide gel electrophoresis, a band was observed which has a mobility similar to that of unglycosylated precursors of GP-2 seen in endodermal cells treated with tunicamycin. This band was not visible in the immunoprecipitates of translation products directed by uninduced F9 RNA. These results suggest that the increase in GP-2 synthesis caused by retinoic acid and dibutyryl cyclic AMP is due to an increase in translatable GP-2 mRNA (messenger RNA). (no Refs)

4. EFFECTS OF RETINOIC ACID ON DIFFERENTIATION OF CHORIOCARCINOMA CELLS IN VITRO (MEETING ABSTRACT).

Chou JY
National Institutes of Health, Bethesda, MD, 20205
Fed Proc; 41(3):683 1982

Choriocarcinoma cells secreted no immunoreactive pregnancy-specific beta 1-glycoprotein (PS betaG) in vitro. These cells can be induced to synthesize low levels of PS betaG by retinoic acid, 8-Bromo-cAMP (8BrcAMP), cholera toxin, methyl isobutyl xanthine (MIX), and 5-Bromo-2'-deoxyuridine (BrdUrd). Simultaneous addition of retinoic acid along with 8BrcAMP, cholera toxin, or MIX gave synergistic induction of PS betaG. Simultaneous addition of retinoic acid and BrdUrd failed to given even additive induction. In addition to stimulating PS betaG production, retinoic acid increased the production of human chorionic gonadotropin (hCG) and its alpha subunits (hCGalpha) by choriocarcinoma cells. Simultaneous addition of retinoic acid along with 8BrcAMP, cholera toxin, or MIX gave additive induction for hCG and hCGalpha. Passage of choriocarcinoma cells in medium containing retinoic acid induced a stable altered phenotype characterized by elevated levels of PS betaG, hCG, and hCGalpha. These retinoid-treated choriocarcinoma cells remained responsive to 8BrcAMP or compounds that increase intracellular cAMP concentrations and to BrdUrd: the production of PS betaG and hCG was greatly stimulated by 8BrcAMP, cholera toxin, or MIX and the production of hCG and hCGalpha was greatly inhibited by BrdUrd. However, the production of hCGalpha was only slightly induced by these cAMP modulators and the production of PS betaG was not increased by BrdUrd. Our data suggest that retinoic acid plus compounds that elevate cAMP concentration may induce differentiation of choriocarcinoma cells in vitro. (no Refs)

5. PROSTAGLANDIN A1 AND E1 INHIBIT THE PLATING EFFICIENCY AND PROLIFERATION OF MURINE MELANOMA CELLS (CLOUDMAN S-91) IN SOFT AGAR.

Bregman MD, Sander D, Meyskens FL
Dept. Internal Medicine, Univ. Arizona, Tucson, AZ, 85721
Biochem Biophys Res Commun; 104(3):1080-1086 1982

The effects of prostaglandin A1 (PGA1), prostaglandin E1 (PGE1), and prostaglandin F2alpha (PGF2a) on melanoma colony forming units (MCFU) was studied in soft agar cultures of murine melanoma cells (Cloudman S-91). In the study, 5,000 cells were plated in the bilayer soft agar system and colonies greater than 60 micron were counted on day 10. While PGA1 and PGE1 inhibited melanoma colony formation in a dose-dependent manner at concentrations as low as 0.5 ug/ml, PGF2a did not inhibit the number of colonies compared to controls. Analysis of the effect of prostaglandins on colony diameters showed that PGF2a had no effect while both PGA1 and PGE1 altered the size distribution of MCFU. The effect of prostaglandins on cyclic (c)AMP and cyclic guanosine monophosphate (cGMP) levels in cells was evaluated. PGE1 was a very potent stimulator of both cAMP and cGMP levels whereas PGF2a and PGA1 were less effective. While PGE1 generated a 70-fold increase in cAMP levels it was only slightly more inhibitory than PGA1 on colony formation. Even though PGF2a was more effective than PGA1 in raising the levels of cAMP and cGMP, it had no effect on in vitro colony growth. The study suggests that the inhibition of Cloudman S-91 murine melanoma cell growth occurs via a non-cyclic nucleotide mechanism. (20 Refs)

6. SECRETION OF PLASMINOGEN ACTIVATOR BY CBA MOUSE TERATOCARCINOMA CELL LINE (H6) DURING TERMINAL DIFFERENTIATION INDUCED BY RETINOIC ACID (MEETING ABSTRACT).

Littlefield JW
Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD
Pediatr Res; 16(4,part2):114A 1982

Mouse teratocarcinoma cell lines provide a model system in which aspects of early embryogenesis can be studied in detail. Differentiation of essentially all H6 teratocarcinoma cells to endoderm-like cells occurs following exposure to retinoic acid (3x10-6M) for even as short a time as a few minutes. During the subsequent 1-2 weeks the H6 cells divide up to 6 times, but then cannot be passaged further. At this point the culture is composed entirely of large flat cells, which soon degenerate. Flow cytometric analysis indicates these cells are not polyploid. Differentiated H6 cells resemble endoderm on the basis of morphology, increased secretion of plasminogen activator, and analogy with other teratocarcinoma cell systems. After 3-4 days of exposure to retinoic acid, plasminogen activator secreted by mass cultures is increased about 5-fold, and then does not increase further. Treatment with dibutyryl cAMP (10-3M) alone does not increase the secretion of plasminogen activator, but when combined with retinoic acid it causes about a 5-fold further increase in secretion. Study of plasminogen activator secretion by individual cells suspended in casein-agarose suggests that retinoic acid, and especially retinoic acid plus dibutyryl cAMP, increases both the number of cells transiently or permanently secreting plasminogen activator and probably also the amount of plasminogen activator secreted per cell. (no Refs)

7. REVERSION OF HUMAN MAMMARY CARCINOMA TO NORMAL EPITHELIAL CELLS (MEETING ABSTRACT).

Hakim AA
University of Illinois at the Medical Center, Chicago, IL
148th National Meeting of the American Association for the Advancement of Science, January 3-8, 1982, Washington, D.C. American Association for the Advancement of Science 182 pp., 1982.

There is a strong relationship between anchorage independence, ie ability to grow unattached, and the tumorigenic properties of cells. The adhesion of cultured fibroblasts or epithelial cells is mediated by fibronectin and/or laminin, respectively. Reduction of cell-surface fibronectin or laminin correlates with the ability of the transformed cells to produce tumor and metastasize. In the present studies, human carcinoma cells (HMCC), and normal mammary epithelial cells (HNMEC) were cultured in presence and absence of graded amounts of fibronectin (Fn), laminin (Ln), dibutyryl-cyclic AMP and adenosine triphosphate (ATP), each alone and combined, for series of subcultures. At each subculture, the cells were examined for the following parameters: Oncogenicity in athymic Nu/Nu mice, agglutinability by concanavalin A, release of plasminogen activator (PA) and glutamyl-transpeptidase (GTase), and by electron microscope. After five subcultures in media supplemented with Fn, Ln and db-cyclic-AMP, HMCC cells progressively lost their oncogenicity and reverted to normal mammary epithelial cell characteristics. Under all experimental conditions, NNMEC cells released statistically non-significant PA and GTase. During reversion of HMCC these released enzyme activities decreased to Nil. (no Refs)

8. IN VIVO AND IN VITRO CHARACTERIZATION OF A TRANSPLANTABLE, SPONTANEOUS MURINE ASTROCYTOMA (MEETING ABSTRACT).

Pilkington GJ, Darling JL, Lantos PL, Thomas DG
Department of Neuropathology, Institute of Psychiatry, London, England
Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh, Scotland. British Association for Cancer Research 78 pp., 1982.

Spontaneous brain tumors of mice are rare, but Fraser found gliomas in 1.5% of mice from the inbred VM strain. Homogenates of tumor-bearing brain when injected intracerebrally into syngeneic mice produced anaplastic astrocytomas. The present paper describes the fine structure and immunohistochemistry of these transplanted tumors. Three cell lines derived from VM tumors have also been maintained in vitro and examined by fluorescence and electron microscopy in order to establish the glial nature of the cells. Immunofluorescence studies using the astrocyte specific markers - glial fibrillary acidic protein and glutamine synthetase - and large extracellular transformation sensitive protein were carried out. Transmission electron microscopy of cell pellets reveals different ratios of microtubules to 10nm filaments in the 3 cell lines; this indicates the degree of cellular differentiation. The effects of dibutyryl cyclic AMP on the surface activity and organelle distribution have also been assessed. Multicellular spheroids have been induced and examined by scanning and transmission electron microscopy in order to elucidate the extent of adhesion between cells. (2 Refs)

REGULATION OF METABOLISM AND CELL FUNCTION BY CYCLIC NUCLEOTIDES

9. RELATIONSHIP BETWEEN CHEMOTAXIS, CHEMOTACTIC MODULATORS, AND CYCLIC NUCLEOTIDE LEVELS IN TUMOR CELLS (MEETING ABSTRACT).

Mokashi S, Delikatny J, Orr W
Pathology Department, University of Manitoba, Winnipeg, Manitoba R3E OW3, Canada
Fed Proc; 41(3):334 1982

Walker 256 carcinosarcoma cells respond to chemotactic stimuli. Since cyclic nucleotides are involved in the chemotaxis of other cells, we have examined the action of cAMP, db cAMP (dibutyryl cAMP) and cGMP (guanosine monophosphate) as chemoattractants, and as modulators of tumor cell movement. We have also studied the effect of a C5-derived chemoattractant and prostaglandins on intracellular cyclic nucleotide levels, and the effect of prostaglandins as modulators of chemotaxis. Neither cAMP nor cGMP was a chemoattractant, but cGMP at low concentrations (10(-9) M) enhanced chemotaxis approx 2-fold, and both cAMP and db cAMP had an inhibitory effect at concentrations greater than 10(-6) M. In response to 2 ED50 chemoattractant, there was approx 1.4-fold rise in intracellular cAMP within 30 seconds which returned to basal levels within 2 minutes. Intracellular cGMP levels became elevated approx 3.5-fold after this time. Incubation of cells with prostaglandin A1 and F2 alpha stimulated chemotaxis at lower concentration (10(-7) M and 10(-9) respectively) and resulted in elevation of cGMP while incubation with PGE2 resulted in inhibition of chemotaxis and a rise in cAMP levels. The results suggest that cAMP and cGMP may be involved in the regulation of tumor cell chemotaxis. (no Refs)

10. RESPONSES OF HUMAN CHONDROSARCOMAS TO N6-MONOBUTYRYL CYCLIC AMP, PROSTAGLANDIN A1 AND SERUM FACTORS (MEETING ABSTRACT).

McCumbee WD, Lebovitz HE
Duke Medical Center, Durham, NC, 27710
Fed Proc; 41(3):736 1982

In cartilage, RNA synthesis is stimulated by serum from intact (Norm) and growth hormone-treated hypophysectomized (GH-Rx) rats but not by serum from untreated hypophysectomized (Hypx) rats. N6-monobutyryl cyclic AMP (MBcAMP) either stimulates (embryonic chick cartilage) or has no effect (rat costal cartilage) on RNA synthesis, whereas prostaglandin A1 (PGA1) profoundly inhibits the synthesis of RNA. The present study investigated the in vitro regulation of [3H]uridine incorporation into RNA of 5 human chondrosarcomas to determine if malignant human chondrocytes are under similar metabolic and hormonal regulation. Uridine incorporation into RNA was stimulated by growth hormone-dependent serum factors in one tumor and by growth hormone-independent factors in 2 tumors. Two tumors were unresponsive to serum stimulation. PGA1 markedly inhibited uridine incorporation into RNA in all 5 tumors (56 to 80%). MBcAMP inhibited uridine incorporation in 4 of the 5 tumors by 32 to 50%. The data indicate that chondrosarcoma cells. This is similar to its effect on normal chondrocytes. MBcAMP regulation differs from that observed in normal chondrocytes: MBcAMP inhibited RNA synthesis in most of the chondrosarcomas studied. Tumor responses to serum factors vary among individual tumors with one responding similarly to normal cartilage and the others not. (no Refs)

11. PROSTAGLANDIN INHIBITION OF PMA INDUCED TUMOR CELL ADHERENCE (MEETING ABSTRACT).

Fantone JC, Varani J
Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, 48109
Fed Proc; 41(3):1078 1982

Tumor cell metastasis is thought to be initiated by the adherence of circulating tumor cells to endothelial cells and/or basement membrane. Several studies have suggested that the local generation of inflammatory mediators can alter tumor cell adhesiveness resulting in increased metastasis at sites of inflammation. Recent studies in our laboratory have demonstrated increased adherence of Walker Carcinosarcoma cells (WCC) to plastic plates, bovine corneal endotheial cells, and nylon fibers when stimulated with trypsinized fragments of C5, f Met-Leu-Phe, and phorbol myristate acetate (PMA). When WCC are pre-treated with PGE2 (10-5M) or 15-Methyl-PGE1 (10-5M) in the presence of theophylline (2.5 x 10-4M) there was 43.4 and 89.1% inhibition of PMA induced adherence to plastic plates. Thromboxane B2 and PGE2 did not significantly alter PMA induced adherence. WCC pretreated with histamine (10-4M), cholera toxin (10-6M), and dibutyryl cAMP (10-5M) showed similar inhibitory effects. These studies demonstrate that the anti-aggregatory prostaglandins 15-M-PGE1 and PGI2 can inhibit tumor cell adherence in vitro through their direct action on tumor cells. These effects may be mediated through alteration of intracelular cAMP levels. (no Refs)

12. EFFECT OF CYCLIC AMP AND CYCLIC GMP ON THE ACTIVITY OF INTERFERON AGAINST HERPES SIMPLEX VIRUS TYPES 1 AND 2, AND VESICULAR STOMATITIS VIRUS.

Stanwick TL, Nahmias AJ
Microbiology Dept., Univ. Health Sciences, Chicago Medical Sch., 3333 Green Bay Road, North Chicago, IL, 60064
Arch Virol; 72(4):247-256 1982

The antiviral action of interferon (IFN) on herpes simplex virus (HSV) Types 1 and 2, and vesicular stomatitis virus (VSV) was examined with respect to the intracellular levels of cyclic adenosine monophosphate (CAMP) and cyclic guanosine monophosphate (CGMP) in human fibroblast (HF) cultures. Interferon by itself increased the intracellular levels of CAMP, but had no effect on the CGMP levels. Inoculation of HF with HSV or VSV, however, decreased the CAMP levels. Also, HSV inoculation elevated the CGMP levels, but VSV did not alter the CGMP levels. When HSV and IFN were added to the HF cultures in various combinations, HSV inhibited the IFN-induced elevation of CAMP and increased the CGMP levels to that characteristically observed with HSV inoculation in the absence of IFN. In contrast, IFN antagonized the VSV-associated decrease in CAMP levels. Although the yields of VSV were unaltered by the presence of CAMP- or CGMP-enhancing compounds, the yields of HSV were decreased with CAMP enhancers and increased with CGMP enhancers. The combination of IFN and CAMP enhancers had an additive effect in reducing the yields of HSV. Neither the CAMP or CGMP enhancers affected the action of IFN on VSV. These studies suggest that the interactions observed between HSV and cyclic nucleotides might account for the relatively refractive nature of HSV, as compared to VSV, toward the antiviral activity of IFN. (Author abstract) (23 Refs)

CYCLIC NUCLEOTIDE METABOLISM: HORMONAL STUDIES

13. RELATIONSHIPS BETWEEN STIMULATED PROLACTIN RELEASE FROM GH CELLS AND CYCLIC AMP DEGRADATION AND FORMATION.

Gautvik KM, Kriz M, Jahnsen T, Haug E, Hansson V
Fysiologisk Institutt, Universitetet i Oslo, Karl Johansgt. 47, Oslo 1, Norway
Mol Cell Endocrinol; 26(3):295-308 1982

The relationship between stimulated prolactin (PRL) release and formation and inactivation of cyclic AMP (cAMP) was studied in clonal strains (GH3 and GH4C1) of PRL producing rat-pituitary tumor cells (GH cells) after treatment with thyroliberin (TRH) and theophylline (TEP). The effect of TRH on adenyl cyclase activity in crude membrane fractions was investigated. Measurements of cAMP hydrolysis gave a non-linear Lineweaver-Burk plot. Changes in phosphodiesterase (PDE) activity caused by treatment with TRH and TEP were observed in the soluble fraction or in homogenates of cells. The enzymic activities in the soluble fraction and in the particulate fraction showed the same kinetics indicating the presence of identical or closely related cAMP PDEs. The maximal effect on PDE was observed at 30 nanomoles TRH. When TEP enhanced the release of PRL, the cells had increased cAMP concentrations and reduced cyclic nucleotide PDE activity. The effect of TRH on cAMP PDE was duplicated by treatment of the cells with high concentrations of cAMP. The effect of TRH on cAMP hydrolysis was dose-dependent, affecting the apparent maximal velocity of the enzyme rather than Km. Results indicated that TRH and TEP cause a rise in cellular concentrations of cAMP via different mechanisms. The highly positive correlation between PRL release and cellular cAMP concentrations support the notion that in GH cells, cAMP is a mediator of PRL release. (24 Refs)

14. STIMULATION OF B16 MELANOMA ADENYLATE CYCLASE ACTIVITY BY MSH RELATED PEPTIDES (MEETING ABSTRACT).

MacNeil S

Department of Human Metabolism and Clinical Biochemistry, Medical School, University of Sheffield, Sheffield, England

Br J Dermatol; 106(1):115 1982

There is now evidence that the melanosome dispersing activity of MSH depends on two 'message' sequences, corresponding to positions 5-9 (Glu-His-Phe-Arg-Trp) and 10-13 (Gly-Lys-Pro-Val NH(2)) of the molecule. As well as this rapid action on amphibian melanophores, MSH and related peptides stimulate melanogenesis both in amphibian skin and in mammalian melanocytes and melanoma cells through stimulation of tyrosinase activity. This action is known to be mediated through cyclic AMP dependent mechanisms. The ability of alpha-MSH, beta-MSH and related ACTH peptides to stimulate an adenylate cyclase enzyme in a highly pigmented B16 melanoma cell line has recently been investigated in our laboratory. The peptides alpha-MSH, beta-MSH 1-39, ACTH 1-24 all stimulated the enzyme to a similar maximal extent and with similar potency, although ACTH 1-39 was slightly less potent. The heptapeptide sequence ACTH 4-10 was, however, only a partial agonist of this enzyme as was an 11 amino acid synthetic MSH preparation. Our data suggest that at least part of the heptapeptide sequence ACTH 4-10 is necessary for the activation of the receptor-linked adenylate cyclase system but that this sequence alone does not contain all of the structural features required for full activation of the enzyme. It could well be that melanoma cells recognize the same two 'message' sequences on the MSH peptide that determine the melanosome dispersing activity and it is possible that both sequences are required for full activation of adenylate cyclase and hence melanogenesis. However, while the sequence ACTH 14-24 reduces the melanosome dispersing action of ACTH 1-13 (alpha-MSH), in the frog melanophore, in our studies using the B16 melanoma, the adenylate cyclase response to ACTH 1-13 was reduced by the sequence ACTH 25-39. Furthermore, melanotrophic potentiating factor (MPF), which by itself failed to activate the B16 adenylate cyclase receptor, had no effect on the response to alpha-MSH whereas it has been reported to potentiate the actions of alpha-MSH in lizard skin and the melanogenic response to alpha-MSH in hamster follicles. (6 Refs)

15. PURIFICATION AND PARTIAL CHARACTERIZATION OF A BONE-RESORPTIVE PROTEIN FROM CANCER ASCITES FLUID.

Humphries DE

Boston University Graduate School, Boston, MA

Diss Abstr Int [B]; 42(6):2341-B 1982

A protein capable of stimulating bone-resorption in vitro has been purified approximately 1250-fold from cancer ascites fluid. An apparent molecular weight of 43,000 daltons was calculated. In terms of its amino acid composition, the bone-resorptive protein is distinguished by its relatively high content of glycine (17%) and proline (11%). Initial biological characterization of the purified ascites protein demonstrated that the protein is as potent as parathyroid hormone in its ability to stimulate bone-resorption in vitro. Both proteins stimulated the release of radioactivity into the medium from bones which were previously labeled with calcium-45 or tritiated proline indicating that both matrix and mineral were solubilized. However, the bone-resorptive protein present in cancer ascites fluid can be distinguished from parathyroid hormone. The molecular weight of the protein purified in this study is approximately four times that of parathyroid hormone and the ascites protein contains no methionine while this amino acid is present in parathyroid hormone. Also, in contrast to parathyroid hormone, stimulation of isolated bone cells with the ascites protein did not increase cAMP levels. Moreover, although parathyroid hormone and the ascites protein increased calcium uptake in isolated bone cells, only the ascites bone-resorptive protein stimulated increased calcium uptake in isolated skin cells. (no Refs)

16. DERANGEMENT OF CYCLIC NUCLEOTIDE SYSTEMS OF MONONUCLEAR BONE MARROW CELLS IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) (MEETING ABSTRACT).

Skala JP, Rogers PC, MacLaren IA

Department of Paediatrics, University of British Columbia, Vancouver, British Columbia, Canada

Pediatr Res; 16(4,part2):215A 1982

Concentrations of both adenosine 3':5' monophosphate (cAMP) and guanosine 3':5' monophosphate (cGMP) were determined during incubations of mononuclear cells isolated from marrows of ALL patients at diagnosis and at subsequent remission periods. Incubations for 1-20 min in the presence of 1-methyl-3-isobutyl-xanthine resulted in equal increases of both nucleotides whereas addition of sodium nitroprusside resulted in large elevations of cGMP only. Both isoproterenol and epinephrine increased markedly concentrations of cAMP in cells (greater than 70% lymphocytes) isolated from patients undergoing remission of ALL. Carbamylcholine addition resulted in slight increases of the cGMP concentration, whereas serotonin exhibited pronounced stimulatory effect upon guanylate cyclase activity. Cells isolated from ALL patients at diagnosis (greater than 90% blasts) exhibited diminished responsiveness of both cyclic nucleotides to hormonal stimuli, the catecholamine activation of adenylate cyclase being almost absent. The sensitivity had reappeared already by 2 weeks after the initiation chemotherapy. Attempts are made to correlate the characteristics of the cAMP and cGMP systems with the growth behavior of the cells in long-term cultures. The techniques may eventually be used to determine the effectiveness of particular therapeutical interventions in individual patients. (no Refs)

CYCLIC NUCLEOTIDE METABOLISM: NON-HORMONAL REGULATORY FACTORS

17. INTERFERON ENHANCES GUANYLATE CYCLASE ACTIVITY IN HUMAN LYMPHOMA CELLS.

Rochette-Egly C, Tovey MG
Unite INSERM 61, Avenue Moliere, 67200 Strasbourg, France
Biochem Biophys Res Commun; 107(1):150-156 1982

Treatment of the human Burkitt lymphoma derived cell line Daudi with electrophoretically pure human interferon alpha (IFA), which causes a rapid increase in the intracellular concentration of cyclic guanosine monophosphate (cGMP), enhances guanylate cyclase activity. The increase in guanylate cyclase activity in IFA-treated cells resembled that induced by 10 milliM sodium azide in in vitro systems. Neither partially purified nor electrophoretically pure IFA had any effect on guanylate cyclase activity in a cell-free system made from Daudi cells, however. These results strongly suggest that activation of guanylate cyclase in IFA-treated cells is mediated via interaction of interferon with its specific cell surface receptor. The increase in cGMP in IFA-treated cells was calcium-dependent and was abrogated by treatment with the cyclo-oxygenase inhibitors aspirin and indomethacin. (17 Refs)

18. THREE ADENYLATE CYCLASE PHENOTYPES IN S49 LYMPHOMA CELLS PRODUCED BY MUTATIONS OF ONE GENE.

Bourne HR, Beiderman B, Steinberg F, Brothers VM
Div. Clinical Pharmacology, Dept. Medicine, Univ. California Sch. Medicine, San Francisco, CA, 94143
Mol Pharmacol; 22(1):204-210 1982

The nature of the phenotypic lesions affecting the guanine nucleotide-binding regulatory component (N protein) of adenylate cyclase in three mutant lines of S49 mouse lymphoma cells were studied. The mutant lines were cyc-, in which the N protein appears functionally absent; unc, in which the N protein's interactions with hormone receptors are impaired; and H21a, in which N appears to be unable to interact with the catalytic unit of adenylate cyclase. Gel filtration showed that the N protein of H21a probably does not differ in size from that of the wild-type. H21a X unc hybrid cells accumulated cyclic AMP in response to cholera toxin, but not isoproterenol or prostaglandin E1, and adenylate cyclase in membranes of the hybrid was phenotypically unc, indicating that it is highly likely that the defects in both mutants are caused by defects in the same gene. Similarly, both the H21a X cyc- and unc X cyc- hybrids failed genetic complementation, indicating that the three parental types bear mutations of the same gene. Results of agonist binding to beta-adrenergic receptors supported the complementation experiments. All three mutations are probably in a structural gene that codes for both the 42,000 and 52,000-dalton subunits of the N protein; this interpretation fits biochemical and genetic evidence from this and earlier studies. (25 Refs)

19. CHANGES IN THE CYCLIC NUCLEOTIDE SYSTEM IN 1,2-DIMETHYLHYDRAZINE INDUCED RAT INTESTINAL ADENOCARCINOMAS.

Loven DP
The University of Iowa, Iowa City, IA
Diss Abstr Int [B]; 42(7) 1982

The cAMP (cyclic AMP) content of the adenocarcinoma was lower than either normal intact intestine or the mucosal layer. The cAMP content of intact intestine distant from any visible lesion in tumor bearing animals was similar to normal intact intestine. The cAMP content of intact intestine adjacent to the adenocarcinoma was similar to the tumor. The (cyclic guanosine monophosphate) cGMP content of the adenocarcinoma was elevated compared to the normal intact intestine, but was unchanged compared to the normal intestinal mucosa. The cGMP content of intact intestine distant from any visible lesions in tumor bearing animals was similar to the normal intact intestine. The cGMP content of intact intestine adjacent to the adenocarcinoma was similar to the tumor. Isoproterenol, carbachol, and PGE2 (prostaglandin E2) caused a greater increase in cAMP in normal colon mucosa compared to the colon adenocarcinoma in vitro. The basal and total adenylate cyclase activity was depressed in broken tumor cell preparations compared to normal colon mucosa. Agents added in vitro similarly affected the cGMP content of colon adenocarcinoma and normal colon mucosa. The basal guanylate cyclase activity of broken cell preparations of colon adenocarcinoma and normal colon mucosa were similar, however, the total guanylate cyclase activity was depressed in the tumor compared to normal colon mucosa. The cAMP phosphodiesterase activity of the colon adenocarcinoma was elevated compared to normal colon muscosa. The cGMP phosphodiesterase activity of the colon tumor was similar to normal colon mucosa. The cAMP dependent protein kinase activity of the colon adenocarcinoma was depressed compared to normal colon mucosa. (no Refs)

20. STUDIES OF THE PLASMA MEMBRANE IN THE DIFFERENTIATION OF NORMAL AND MALIGNANT THYMOCYTES.

Casteel N
University of Health Sciences/The Chicago Medical School, Chicago, IL
Diss Abstr Int [B]; 42(10):4009-B 1982

The role of the plasma membrane in the differentiation and activation of thymocytes and how cyclic nucleotide-related enzymes are altered with the progress of leukemia was investigated. Enzyme levels of thymocytes of normal, preleukemic and leukemic mice were compared. Both virally-induced leukemia of AKR mice and chemically-induced leukemias of Swiss mice injected with 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC) were studied. While total AMPase activity appeared erratic, 5'-nucleotidase dropped significantly while alkaline phosphatase activity rose dramatically in the thymocytes of both leukemic models. Preleukemic thymocytes showed intermediate values. This is in contrast to normal developmental changes, where the activities of 5'-nucleotidase and alkaline phosphatase rise in parallel. Phosphodiesterase activity of the thymocytes of these mice also became elevated with leukemia, confirming that the enzymic changes that would lead to lowered cAMP levels and increased proliferation, ie, lowered 5'-nucleotidase and elevated phosphodiesterase activities are controlling factors in the development of both virally-induced and chemically-induced leukemias. (no Refs)

21. ISOLATION AND CHARACTERIZATION OF NEUROBLASTOMA CLONAL VARIANTS.

Lombardo JM
State University of New York, Downstate Medical Center, New York, NY
Diss Abstr Int [B]; 42(6):2315-B 1981

Two spontaneously arising variant clones were selected from the N18 neuroblastoma cell line solely on the basis of their flattened morphology and tight adherence to the culture flask.

Two other clones having the round loosely adherent morphology typical of the parent line were also obtained. In addition, revertant clones exhibiting the flat morphology were isolated from one of the round clones after prolonged cultivation and revertant clones exhibiting the round parental morphology were isolated from one of the flat variant clones. The flat variant clones have slower growth rates in culture, lower cloning efficiences in suspension and reduced acetylcholinesterase inducibility when compared with either the parent N18 line or the round cell clones. Cells of both morphologic types have high levels of plasminogen activator and are tumorigenic although the variants have a slower growth rate in vivo consistent with their slower growth rate in culture. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of total protein from the two cell types shows that the flat variants have increased amounts of a 200,000 molecular weight polypeptide identified as myosin heavy chain by peptide mapping. Intracellular cAMP levels in flat variants and flat cell revertants were increased from two-to-five-fold over those in clones representative of the five-parent line and in round cell revertants. Treatment with the phosphodiesterase inhibitor RO20-1724 increased cAMP levels and reduced suspension cloning efficiency without altering doubling time or attached cloning efficiency. Increasing cAMP levels of two of the round cell clones by treatment with the phosphodiesterase inhibitor caused increased flattening of the cell body and increased myosin heavy chain content. These data strongly suggest that the ability of these neuroblastoma cells to grow in suspension is cAMP dependent and that a relationship between cAMP, myosin heavy chain and morphology exists. (no Refs)

22. DIRECT INHIBITION OF THE CATALYTIC PROTEIN OF ADENYLATE CYCLASE AT THE ADENOSINE P SITE (MEETING ABSTRACT).

Florio VA, Ross EM
University of Texas Health Science Center, Dallas, TX, 75235
Fed Proc; 41(3):1408 1982

Both adenosine and the P site agent 2',5'-dideoxyadenosine (DDA) reversibly inhibit the activity of the catalytic protein (C) of adenylate cyclase either in a resolved C fraction from hepatic plasma membranes or in plasma membranes of cyc- S49 lymphoma cells. Both preparations are free of the GTP(guanosine triphosphate)-binding regulatory protein, G/F. Inhibition by DDA is insensitive to 0.4 mM 1-methyl-3-isobutylxanthine or adenosine deaminase (2.5 IU/ml). The low basal activity of C in the presence of Mg++ is slightly inhibited. Mn++-stimulated activity (6-10 x basal) is inhibited up to 40%. Further reversible stimulation by Mn++ plus forskolin (10-30 x Mn++) or quasi-irreversible stimulation by GTP-gamma-S-preactivated G/F (5-20 x Mn++) are completely blocked by Ado or DDA. There is no effect of submaximal amounts of DDA on the concentrations of Mn++, forskolin, or GTP-gamma-S-preactivated G/F needed for activation of C, nor is there an effect of any activator on the concentration dependence of inhibition by DDA. DDA does not alter the rate of activation of G/F by GTP-gamma-S nor does it promote dissociation of the stable G/F-C complex formed in the presence of GTP-gamma-S. DDA is not competitive with substrate, as shown by others. These data suggest that DDA acts directly on C, but that it does not bind or regulate C when activating ligands are absent. (no Refs)

23. ADENYLATE CYCLASE COUPLED BETA2-ADRENERGIC RECEPTORS IN A SMOOTH MUSCLE TUMOR CELL LINE (MEETING ABSTRACT).

Garmer J, Cornett LE, Norris JS
Departments of Medicine and Physiology, University of Arkansas for Medical Sciences, Little Rock, AR, 72205
Fed Proc; 41(3):1511 1982

The DDT1 MF-2 cell line was established by explantation from an androgen-estrogen induced leiomyosarcoma of the ductus deferens of a Syrian hamster. Since these cells morphologically resemble smooth muscle cells and contain alpha 1-adrenergic receptors, we chose to examine them for the presence of beta-adrenergic receptors. Binding of [3H]-dihydroalprenolol (DHA) to membranes of DDT1 cells grown in suspension culture was of high affinity, saturable, stereoselective and reversible. The dissociation constant for DHA was 0.8 nM with site number of 425 fmoles/mg protein. The order of agonist competition was isoproterenol greater than epinephrine greater than norepinephrine consistent with a beta 2-receptor interaction. Furthermore, Zinterol, beta 2-selective, was more effective than Metoprolol, beta 1-selective in competing for the DNA binding site. Also, the DDT1 cell beta 2-adrenergic receptor is functionally linked to adenylate cyclase as basal cyclic AMP accumulation (112pmol/10(6) cells/10') was stimulated by 10 uM isoproterenol (402pmol/10(6) cells/10'). Stimulation by isoproterenol was inhibited by 10uM propranolol (102pmol/10(6) cells/10'). In conclusion, the presence of both alpha 1-and beta 2-adrenergic receptors make the DDT1 cell an ideal model in which to study the actions of catecholamines on smooth muscle. (no Refs)

24. EFFECT OF ACTIVATORS OF CAMP ACCUMULATION ON VARIOUS STAGES OF GENOME EXPRESSION DURING ACUTE RADIATION INJURY. COMMUNICATION 3. COMPARATIVE ANALYSIS OF PROPERTIES OF RNA SYNTHESIZED IN ISOLATED NUCLEI OF LIVER AND SPLEEN FROM IRRADIATED RATS PRETREATED WITH SEROTONIN.

Tsudzevich BA, Galkina LA, Kucherenko NE
Biology Faculty, T. G. Shevchenko State Univ., Kiev, USSR
Radiobiologiia; 22(3):379-383 1982

The mechanism of radioprotective effect of serotonin was studied in cell nuclei isolated from the liver and spleen of irradiated rats subjected to whole-body irradiation with 800 rads. Fractionation of RNA synthesized in the isolated cell nuclei showed that irradiation inhibited the transcription, processing, and release of messenger RNA. Activation of cAMP system (pretreatment with serotonin) increased the radioresistance of RNA synthesis. (10 Refs) (In RUS)

25. CHOLERA TOXIN STIMULATION OF HUMAN MAMMARY EPITHELIAL CELLS IN CULTURE.

Stampfer MR
Peralta Cancer Res. Inst., Peralta Hosp., 3023 Summit St., Oakland, CA, 94609
In Vitro; 18(6):531-537 1982

Addition of cholera toxin to human mammary epithelial cultures derived from reduction mammoplasties and primary carcinomas greatly stimulated cell growth and increased the number of times the cells could be successfully subcultured. Other agents known to increase intracellular cAMP levels were also growth stimulatory. The increased growth potential conferred by cholera toxin enhances the usefulness of this cell culture system. (Author abstract) (17 Refs)

26. COMPARISON OF OPIATE INHIBITION OF ADENYLATE CYCLASE ACTIVITY IN NEUROBLASTOMA N18TG2 AND NEUROBLASTOMA X GLIOMA NG108-15 HYBRID CELL LINES.

Law PY, Koehler JE, Loh HH

Dept. Pharmacology, Univ. California, San Francisco, San Francisco, CA, 94143

Mol Pharmacol; 21(2):483-491 1982

The ability of opiate agonists, in particular the opioid peptides, to regulate adenylate cyclase (AC) in the N18TG2 cells is compared with the opiate inhibition of the AC in NG108-15 cells. Opioid peptides and opiate alkaloids, with exception of morphine, inhibited the AC activity in the neuroblastoma N18TG2 cells. Some distinct differences in opiate inhibition of AC activity were observed in the N18TG2 and NG108-15 cells suggesting that there are differences in the molecular events which lead to opiate inhibition of AC activity in the two cell lines. (21 Refs)

27. POTENTIATION OF OPIATE ACTION IN NEUROBLASTOMA N18TG2 CELLS BY LIPID INCORPORATION.

Law PY, Nicksic TD, O'Rourke MA, Koehler JE, Herz A, Loh LL

Dept. Pharmacology, Univ. California, San Francisco, San Francisco, CA, 94143

Mol Pharmacol; 21(2):492-502 1982

Lipids were incorporated into the cellular membrane of N18TG2 cells, and the subsequent effect on opiate receptor binding and opiate inhibition of adenylate cyclase activity was investigated. A concentration- and time-dependent incorporation of sulfatide by N18TG2 cells was observed. At the maximal concentration of cerebroside sulfate used (67 uM) the opiate receptor activity in N18TG2 cells approached that of NG108-15 cells. Of all the phospholipides tested only phosphatidylcholine produced a potentiation of the opiate effect. (40 Refs)

CYCLIC NUCLEOTIDE METABOLISM: GENERAL STUDIES

28. CONCENTRATION OF CYCLIC NUCLEOTIDES IN PERIPHERAL BLOOD LEUKOCYTES OF CHILDREN WITH ACUTE LEUKEMIA.

Khakimov ShK

Lab. Clinical Biochemistry, Inst. Pediatrics, Moscow, USSR

Pediatriia; (6):39-40 1982

Concentrations of cyclic nucleotides in peripheral blood leukocytes were determined in 89 children (3-15 yr old) with acute lymphoblastic leukemia. The concentrations of cAMP and cGMP showed significant increases during the acute period and during the medullary recurrence. Polychemotherapy resulted in progressive decreases in the concentrations. (9 Refs) (In RUS)

29. AGE-RELATED CHANGES IN CAMP- AND PROSTAGLANDIN-CONTENT IN THE BRAIN AND LYMPHOCYTES OF C3H MICE WITH SPONTANEOUS BREAST CANCER.

Busse E, Helmholz M, Magdon E

Abt. fur Angewandte Tumorbiologie, Charite-Geschwalstklinik, Schumannstrasse 20-21, DDR-104 Berlin, E. Germany

Onkologie; 4(6):328-330 1981

Age-caused concurrent changes in the activation of the adenylate-cyclase activity by dopamine in the diencephalon and the stimulation of the basal level of cAMP by isoproterenol in the lymphocytes were demonstrated in the spontaneously tumor producing C3H mice. These changes may be related to the occurrence of tumors in these animals. The abovementioned changes in the cAMP system were not found in AB mice. By treating the adult C3H mice with substances which increase the cAMP level, it was possible to achieve a renewed stimulation of the cAMP system as seen in young mice. With this treatment the spontaneous tumor-induction rate was also reduced. In the adenocarcinomas of the C3H mice a decrease in the level of prostaglandin E and an increase in the level of prostaglandin F2alpha was observed. (Author abstract) (13 Refs) (In GER)

30. ATP UPTAKE BY MOUSE LYMPHOMA CELLS.

Garcia De Castro MF, Ayad SR

(c/o Ayad) Dept. Biochemistry, Univ. Manchester, Manchester M13 9PL, England

Anticancer Res; 2(1/2):41-45 1982

P388 mouse lymphoma cells showed little response to prostaglandin E1 (PGE1) in the presence of 3-isobutyl-1 methyl xanthine (IBMX) as a phosphodiesterase inhibitor in terms of cyclic (c)AMP production. By contrast CH23 Chinese hamster fibroblasts and PCM3 cells (a hybrid of the preceding two cell lines) readily responded. Endogenous ATP concentration in P388 cells was shown to be low. The addition of ATP in the presence of IBMX into the incubation medium caused an elevation of the intracellular pool of ATP and the cells responded to PGE1 by increase in cAMP production. The ATP effect was specific and could not be obtained by substitution with guanosine triphosphate. The ATP analogue, AMP-PCP, which is resistant to enzymatic hydrolysis, could produce an effect similar to that of ATP. Intracellular ATP concentration could be lowered by incubation with iodoacetate and KCN. This treatment caused a marked decrease of cAMP whereas ethionine was without effect on intracellular ATP. (21 Refs)

31. EFFECT OF RADIATION ON INDUCED SYNTHESIS OF TYROSINE AMINOTRANSFERASE IN LIVER OF RAT EMBRYO.

Slozhenikina LV, Fialkovskaia LA, Kuzin AM

Inst. Biological Physics, Pushchino, USSR

Radiobiologiia; 22(3):383-386 1982

Effect of radiation on the induced synthesis of tyrosine aminotransferase (TAT) was studied in the liver of 20-day rat embryos irradiated in utero with 600 rads. Increase of TAT activity 4 hr after irradiation was indicative of the de novo synthesis of the enzyme. The increase of TAT activity was correlated with the increase of the level of endogenous cyclic AMP. (12 Refs) (In RUS)

32. CYCLIC NUCLEOTIDE CONTENT OF THE CEREBROSPINAL FLUID OF CHILDREN WITH ACUTE LEUKEMIA.

Khakimov ShK, Gerasimova IuV
Res. Inst. Pediatrics, Moscow, USSR
Vopr Okhr Materin Det; 27(6):46-48 1982

Contents of cyclic AMP (cAMP) and cyclic guanosine monophosphate (cGMP) in cerebrospinal fluids (CSF) were studied in 62 children (3-15 yr old) with acute leukemia (AL). Among these children, 23 had clinical manifestations of neuroleukemia. The children with neuroleukemia had a significant decrease in CSF level of cAMP (16.6 picomoles/mg, compared with 32.71 picomoles/mg in children in remission) and increase in CSF level of cGMP (9.71 picomoles/mg, compared with 6.28 picomoles/mg in children in remission). Intrathecal administration of cytostatic agents resulted in gradual normalization of CSF levels of cAMP and cGMP. (7 Refs) (In RUS)

33. A COMPARISON OF THE VALUE OF SERUM ENZYME LEVELS AND URINE CYCLIC NUCLEOTIDE EXCRETION AS MARKERS FOR CANCER CHEMOTHERAPY (MEETING ABSTRACT).

Skillen AW
University Department of Clinical Biochemistry, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, England
Tenth International Symposium on Clinical Enzymology, April 21-24, 1982, Padua, Italy International Society for Clinical Enzymology; :A1 1982

Serum enzymes and urine cGMP (guanosine monophosphate) excretion have proved of value in monitoring the response to chemotherapy of some 100 patients with ovarian cancer. In general, the levels of all four parameters can be correlated with the progress of the disease, regression being accompanied by a reduction in urine cGMP and a lowering of serum enzyme levels. Tumor reactivation and recurrence is usually accompanied by, and in many cases preceded by, increased levels of all four parameters. The correlation with tumor burden is not good and liver disease can produce elevated levels of urine cGMP as well as increased serum enzyme activity. Decisions as to tumor response can only be made on the basis of serial measurements. As the mechanism responsible for elevations of the serum enzymes and the urine cGMP are different comparison of all four parameters can help to elucidate the cause of altered levels. Examination of serial changes in selected patients with ovarian cancer will illustrate the value of these measurements. (no Refs)

PROTEIN KINASE STUDIES

34. INHIBITION OF MOUSE MASTOCYTOMA PROTEIN KINASES BY AMILORIDE.

Ralph RK, Smart J, Wojcik SJ, McQuillan J
Dept. Cell Biology, Univ. Auckland, Auckland, New Zealand
Biochem Biophys Res Commun; 104(3):1054-1059 1982

In recent studies on the control of growth of PY815 mouse mastocytoma cells by cyclic (c)AMP, it was found that amiloride (AM; a potent inhibitor of Na+ transport) inhibited both Type I and II cAMP-dependent protein kinases prepared from PY815 cells. These studies suggest that AM may affect growth by inhibiting protein phosphorylation and hence cation transport. Increasing concentrations of AM or ouabain progressively inhibited growth at concentrations in excess of 1 x 10(-5) M, indicating a requirement for Na+ transport for continuous growth. In an in vitro assay, increasing concentrations of AM inhibited histone phosphorylation by type I and II cAMP-dependent protein kinases prepared from mastocytoma cells. Histone phosphorylation was inhibited over approx the same AM concentration range that affected growth. Ouabain was without effect. AM (1 milliM) did not affect ATP-45Ca++ uptake by membranes from mastocytoma cells and incubated with azide and oxalate to eliminate effects of residual mitochondria. AM did not prevent the stimulation of 45Ca++ uptake that resulted when the membranes were first preincubated with type I or II protein kinases. Thus, pre-phosphorylating isolated membranes appeared to activate a new route for 45Ca++ transport and inhibiting the kinase with AM prevented formation of this new Ca++ channel. Results suggest that AM-sensitive ion transport channels in cells may be controlled by protein phosphorylation which alters the availability of intracellular cations to affect growth. (22 Refs)

35. INHIBITORY EFFECT OF SOMATOSTATIN ON BASAL AND VASOACTIVE INTESTINAL PEPTIDE-STIMULATED CYCLIC AMP PRODUCTION AND CYCLIC AMP-DEPENDENT PROTEIN KINASE ACTIVITY IN HELA CELLS.

Prieto JC
Departamento de Bioquimica, Facultad de Medicina, Alcala de Henares, Madrid, Spain
FEBS Lett; 144(1):173-176 1982

The effect of somatostatin (SST) on cyclic AMP (cAMP) production and cAMP-dependent protein kinase (PK) activity in HeLa cells was studied and the antagonistic relationship between vasoactive intestinal peptide (VIP) and SST on the regulation of cAMP and cAMP-dependent PK was investigated. VIP stimulated cAMP production in a dose-dependent manner. Half-maximal stimulation was observed at 2.5 +-0.3 nanoM (nM) VIP and maximal at greater than 10 nM. SST at 1 uM significantly inhibited basal and VIP-stimulated cAMP levels. Basal cAMP was inhibited by 40% at 1 uM SST whereas the increment produced by a maximally effective VIP concentration was reduced by 60%. The inhibitory effect of SST was dose-dependent. Both basal and VIP (30 nM)-stimulated cAMP production was progressively inhibited by increasing concentrations of SST, significant decreases being observed at 1 uM. A slight but significant effect on PK basal activity was observed; 1 uM SST inhibited by 60% the increase of the PK activity ratio elicited by 30 nM VIP. Results demonstrated that HeLA cells have specific receptors for SST, but it is not clear whether SST acts by first interacting with a membrane site or after penetrating into the cell. (24 Refs)

OTHER RELATED STUDIES

36. IDENTIFICATION OF CA++-CALMODULIN DEPENDENT ENZYME ACTIVITIES IN AN INSULIN-SECRETING TUMOUR.

Penn EJ, Hutton JC, Hales CN
Dept. Clinical Biochemistry, New Addenbrooke's Hosp., Hills Road, Cambridge CB2 2QR, England
Biochem Soc Trans; 10(4):266-267 1982

The nature of the major calmodulin-binding proteins was investigated in a transplantable rat islet-cell tumor. The eluted proteins, obtained by affinity chromatography from post-microsomal supernatant, representing 1% of the total tissue protein, were distributed among a large number of different peptides. The association between calmodulin and tumor proteins could be reversed by trifluoperazine and required Ca++ (but not Mg++). Investigation of the eluted protein fraction indicated the presence of myosin light-chain kinase, cyclic nucleotide phosphodiesterase, and phosphorylase kinase. Further investigation of myosin light-chain kinase activity indicated a relationship to enzyme from other previously described nonmuscle and smooth muscle cells. The observation that insulin-secretory tissue contains myosin light-chain kinase activity, taken together with the previously reported presence of actin and myosin, is consistent with the involvement of Ca++-regulated contractile processes in the secretory mechanism. (19 Refs)

37. QUANTITATIVE BONE HISTOMORPHOMETRY IN HUMORAL HYPERCALCEMIA OF MALIGNANCY: UNCOUPLING OF BONE CELL ACTIVITY.

Stewart AF, Vignery A, Silverglate A, Ravin ND, LiVolsi V, Broadus AE, Baron R

Yale Univ. Sch. Medicine, 333 Cedar St., New Haven, CT, 06510

J Clin Endocrinol Metab; 55(2):219-227 1982

Humoral hypercalcemia of malignancy (HHM) results from elaboration by tumors of a circulating bone-resorbing factor(s). The specific mechanism responsible for this bone resorption is poorly understood, and no comprehensive study employing quantitative histomorphometric analyses of bone biopsies obtained from living patients with HHM has been reported. We describe bone histology and quantitative bone histomorphometry in bone biopsies obtained from seven patients defined biochemically (elevated nephrogenous cAMP excretion) and histologically (no tumor in biopsy sample) as having HHM. These biopsies are compared to biopsies from nine patients with primary hyperparathyroidism (HPT). Compared to patients with HPT, those with HHM displayed (mean +- SD) greater osteoclastic activity (osteoclast surface, 8.6 +-6.1% vs 2.7 +-1.5%; p less than 0.001) and more frequent empty lacunae (9.2 +-4.0% vs 5.8 +-3.0%; p less than 0.01), but markedly reduced osteoblastic surface (2.5 +-3.1% vs 13.8 +-7.0%; p less than 0.001), osteoid surface (12.9 +-11.9% vs 42.0 +-15.0%; p less than 0.001), and osteoid vol (0.3 +-0.3% vs 1.3 +-1.0%; p less than 0.01). These findings directly confirm the presence of humorally mediated bone resorption and indicate a striking uncoupling of osteoclast and osteoblast activities in bone from patients with HHM. These findings are in sharp contrast to those in HPT patients, where osteoclast and osteoblast activities are tightly coupled, and net skeletal calcium loss is minimal. This uncoupling provides a mechanism for the marked skeletal calcium losses observed in patients with HHM. (Author abstract) (32 Refs)

38. IMMUNOGENICITY CHANGES INVERSELY WITH IMMUNOSENSITIVITY DURING IN VITRO DIFFERENTIATION OF HUMAN TERATOCARCINOMA (MEETING ABSTRACT).

Dewolf WC, Carroll PG
No affiliation given
77th Annual Meeting of the American Urological Association, Inc., May 16-20, 1982, Kansas City, Missouri. American Urological Association Missouri. American Urological Association 254 pp., 1982.

The in vitro teratocarcinoma (TC) model provides a unique setting for analysis of developmental regulation in terms of embryonic and malignant gene expression; this is true because, in contrast to other kinds of tumors, it has the potential to differentiate into tissues derived from all three primary germ layers. In this study, the effects of in vitro differentiation were measured in terms of two fundamental cellular immune properties of tumor cells ie immunogenicity and immunosensitivity. Four characterized human TC cell lines were used. In vitro differentiation was induced by cell culture in the presence of retinoic acid with or without cAMP. TC immunogiuecity was measured by mixed tumor-lymphocyte assay. Results are expressed as mean triplicate values of incorporated 3HTdR (thymidine) by autologous or allogeneic responder lymphocytes. Immunosensitivity was assayed by release of 51Cr from TC target cells upon lysis by activated NK (natural killer) cells; likewise target specificities were checked by cold target inhibition. All 51Cr release assays were performed at multiple E:T ratios. The results showed 1) immunogenicity increased with differentiation and was not associated with HLA-DR expression, 2) immunosensitivity decreased with differentiation, 3) the TC CML target specificity is on all TC cells but not on lymphocytes or lymphoblastoid cells. This work supports the overall concept that the natural killing mechanism functions as a primitive immune system to form a cell mediated resistance barrier against the outgrowth of certain undifferentiated tumor cells. (no Refs)

39. IDENTIFICATION AND CHARACTERIZATION OF A 200,000 DALTON TUMOR ANTIGEN OF AVIAN SARCOMA VIRUS TRANSFORMED BALB/C CELLS (MEETING ABSTRACT).

Parsons SJ, McCarley DJ, Parsons JT, Benjamin DC
University of Virginia, Charlottesville, VA, 22901
Fed Proc; 41(3):555 1982

BALB/c bearing mice tumors induced by avian sarcoma virus (ASV) produce antibodies which immunoprecipitate the viral transforming protein, pp60src, and in some cases, antibodies to phosphoproteins with Mr ranging from 110 to 250 kd. Monoclonal antibodies were produced and used to determine whether these phosphoproteins either play a role in tumor rejection or are associated with pp60src, a known kinase. One monoclonal antibody specifically immunoprecipitates a major polypeptide of 200 kd and two minor polypeptides of 18 and 15 kd. The 200 kd peptide (p200) is found in both normal and transformed mammalian cell lines and chicken embryo fibroblasts (CEF). P200 from normal CEF appears to be slightly smaller in size and either less abundant or less highly phosphorylated than its transformed counterpart. Immunoaffinity purified ASV-BALB/c extracts contain these three polypeptides which, along with several other minor proteins, become phosphorylated during a cyclic nucleotide independent 'autophosphorylation' reaction. (no Refs)

AUTHOR INDEX WITH CITATION NUMBERS

Ayad SR 30
Baron R 37
Beiderman B 18
Benjamin DC 39
Bourne HR 18
Bregman MD 5
Broadus AE 37
Brothers VM 18
Busse E 29
Carroll PG 38
Casteel N 20
Cho-Chung YS 1
Chou JY 4
Chung AE 3
Cornett LE 23
Custer RP 2
Darling JL 8
Delikatny J 9
Dewolf WC 38
Durkin ME 3
Fantone JC 11
Fialkovskaia LA 31
Florio VA 22
Galkina LA 24
Garcia De Castr 30
Garmer J 23
Gautvik KM 13
Gerasimova IuV 32
Hakim AA 7
Hales CN 36
Hansson V 13
Haug E 13
Helmholz M 29
Herz A 27
Huang FL 1
Humphries DE 15
Hutton JC 36
Jahnsen T 13
Khakimov ShK 28
Khakimov ShK 32
Koehler JE 26
Koehler JE 27
Kriz M 13
Kucherenko NE 24
Kuzin AM 31
Lantos PL 8
Law PY 26
Law PY 27
Lebovitz HE 10
LiVolsi V 37
Littlefield JW 6
Loh HH 26
Loh LL 27
Lombardo JM 21
Loven DP 19
MacLaren IA 16
MacNeil S 14
Magdon E 29
McCarley DJ 39
McCumbee WD 10
McQuillan J 34
Meyskens FL 5
Mokashi S 9
Nahmias AJ 12
Nicksic TD 27
Norris JS 23
O'Rourke MA 27
Orr W 9
Parsons JT 39
Parsons SJ 39
Penn EJ 36
Pilkington GJ 8
Prieto JC 35
Ralph RK 34
Ravin ND 37
Rochette-Egly C 17
Rogers PC 16
Ross EM 22
Sander D 5
Schaefer FV 2
Silverglate A 37
Skala JP 16
Skillen AW 33
Slozhenikina LV 31
Smart J 34
Sorof S 2
Stampfer MR 25
Stanwick TL 12
Steinberg F 18
Stewart AF 37
Thomas DG 8
Tovey MG 17
Tsudzevich BA 24
Varani J 11
Vignery A 37
Wojcik SJ 34

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